ANTIDIABETIC STUDIES OF THE AQUEOUS EXTRACTS OF GINGER (*Zingiber officinale*) AND CINNAMON (*Cinnamonum zeylanicum*) AND THEIR COMBINATION IN EXPERIMENTAL RATS

BY

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Declaration

I, Hauwa'u Sa'ad hereby declare that this work is the product of my own research efforts undertaken under the Supervision of Dr. A. J. Alhassan, has not been presented elsewhere for the award of a degree or certificate. All sources have been duly acknowledged.

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Certification

This is to certify that the research work for this dissertation and subsequent write up by Hauwa'u Sa'ad, (SPS/13/MBC/00021) were carried out under my supervision.

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To all my colleagues I say Best of Luck in all your endeavors.

Dedication

I dedicate this work to myself for finally having finished writing this research dissertation. Alhamdulillah.

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Abstract

This research was conducted to evaluate the hypoglycemic and hypolipidemic properties of aqueous extract of Cinnamon (*Cinnamonum zeylanicum*) bark; Ginger (*Zingiber officinale*) rhizome and a combination of the two on Alloxan induced diabetic rats. It also studied the effect of the aqueous extracts on kidney function parameters. Thirty two rats were grouped into 8 groups of four rats each. Group I was the normal control, Group II was administered 200mg/kg aqueous extract of ginger, Group III was administered 150mg/kg aqueous extract of Cinnamon. Group IV the diabetic control group, Group V was a diabetic group administered 200mg/kg aqueous extract of ginger, Group VI is the group administered 150mg/kg aqueous extract of Cinnamon, Group VII was a diabetic group administered a combination of Ginger and Cinnamon aqueous extract in the ratio 4:3 respectively, at a dose of 200mg/kg of ginger and 150mg/kg of cinnamon, Group VIII was a diabetic group treated with metformin 500mg/kg. The animals were treated with the aqueous extracts of ginger and cinnamon, and metformin for 14 days while monitoring the fasting blood glucose at 3-day intervals. The treatment was withdrawn after 14 days and blood glucose sampling continued for 7days at 3 day intervals before the sacrifice. The animals were then sacrificed after 21 days and blood collected was used for analysis of lipid profile and renal function markers. The fasting blood glucose level was found to be significantly($P<0.05$) decreased in all groups treated with the aqueous extracts, with the highest hypoglycemic activity seen in group VII compared to the diabetic group. Hypolipidemic activities were also seen in groups V, VI, and VII with significant ($P<0.05$) decrease in these groups treated with the aqueous extracts of ginger, cinnamon and a combination of both compared to the diabetic control. The electrolytes, Urea and Creatinine levels were found to be unaffected by the treatment on all the groups, a comparison between the treated groups and untreated one showed no significant difference ($P>0.05$). The findings of this study suggests that Ginger rhizome, cinnamon bark and their combination may be used in the management of diabetes mellitus, although further human studies need to be carried to confirm their potential benefits in diabetes management.

CHAPTER ONE

INTRODUCTION

1.1 Herbal medicine

Herbal medicine is very popular worldwide and it has been used as a source of medication since ancient times even before the introduction of the synthetic pharmaceutical drugs. However, some people regard herbal medicine with skepticism especially those in mainstream healthcare delivery system. It is perceived as unsafe, under-developed and not well documented in addition to its possible side-effects and adverse reactions. Traditional medicine is very important in modern medicine as it showcases many readily available plants that offer a great potential for the discovery of new drugs , while many others are yet to be discovered (Hajiaghaalipour *et al.*, 2015; Liu *et al.*, 2015).

In order to encourage people to accept herbal medicine, researchers are undertaking scientific investigations to provide evidences for its efficacy, safety and the possible mechanisms of action. Plants are generally perceived to be safe, but there are plant materials which are hazardous to human being, hence the need to conduct toxicity studies prior to human consumption (Ahmad *et al.,* 2014; Ahmad *et al.,* 2015). The misuse of conventional medicines such as antibiotics has resulted in the development of resistance in many infectious organisms. Thus, herbal preparations can be more effective than conventional medicines and their non-toxic nature means that they can be administered over long periods (Vinothapooshan and Sundar 2010).

1.2 Cinnamon

Cinnamon is mainly known as a spice in most western countries, but it has an old history of been used as a herbal medicine in Asia. It is obtained from the inner bark of trees known as *Cinnamomum zeylanicum* ,a tropical evergreen plant (Ranasinghe et *al.,* 2013). Animal and invitro studies carried out since the early 1990's have indicated that cinnamon may mimic insulin effects, hence improving glucose utilization (Qin *et al.,* 2004; Verspohl *et al.,* 2005). Hwa *et al.,* (2011) reported cinnamon as one of the spices used in the early treatment of chronic bronchitis. Furthermore cinnamon is used in other traditional practices for the treatment of impotence, frigidity, dyspnea, eye inflammation, rheumatism, vaginitis as well as wounds and toothaches (Khan *et al.,* 2003). Novel discoveries are being made on cinnamon's potential through modern research and cutting-edge technologies. These studies showed that cinnamon has hypoglycemic and cholesterol lowering activities (Khan *et al.,* 2003). It was also discovered that a polyphenolic polymer isolated from cinnamon have insulin-like and antioxidant activities *in vitro* (Anderson *et al.,* 2003). Recent studies from Anand *et al.,* (2010) found that *in vitro* incubation of cinnamon extract led to enhanced insulin release while Patel *et al,.* (2012) listed cinnamon as an antidiabetic medicinal plant with insulin mimetic property.

1.3 Ginger

Ginger (*Zingiber officinale*) is one of the most widely consumed spices used for the flavoring of food worldwide (Li *et al*., 2012). It is used for cooking and treating a host of ailments throughout Asia, especially in India and China, for over 5000 years. It can be consumed as a fresh or dried root and is often prepared in teas, soft drinks, and breads. (Khulood, 2014).

1.4 Diabetes mellitus

Diabetes mellitus is the most common metabolic and endocrine disorder worldwide. It is linked to disturbances in carbohydrates, fats and proteins metabolism and is especially important because the global prevalence of diabetes is projected to rise in coming years (Chen *et al.*, 2011). WHO calculates that 347 million people around the world suffer from diabetes, that this number will double by 2030, and that 80% of diabetics live in developing countries (WHO, 2012).

Diabetes mellitus is a chronic disorder of glucose metabolism resulting from dysfunction of pancreatic beta cells and insulin resistance (Abdul Rahim, 2009)

Diabetes mellitus is characterized by hyperglycemia due to insufficiency of secretion or action of endogenous insulin. Symptoms include polyuria (frequent urination), polydypsia (increased thirst) and polyphagia (increased hunger). Although the etiology of the disease is not well defined; viral infection, autoimmune disease, and environmental factors may be some of the causes (Wild *et al., 2004*). Plant remedies have been used for centuries for the treatment of diabetes mellitus but only a few of these plants have been scientifically evaluated (Gwarzo *et al.*, 2010). The plants products used in the management of diabetes mellitus improve lipid metabolism and antioxidant status of patients in addition to its hypoglycemic activities (Bailey and Day, 1989).

1.5 Statement of the problem

Diabetes mellitus is a serious global health problem. The disease affects both genders and all age groups, so there is a serious concern about its control and treatment. (Abdul Rahim, 2009). According to Sobnigwe *et al.*, (2001) the prevalence of diabetes mellitus is on the increase in African communities due to ageing of the population and drastic lifestyle changes accompanying urbanization and westernization.

The goal of management of diabetes is to avoid or minimize chronic diabetic complications, as well as to avoid acute problems of hyperglycemia (Itelima *et al.,* 2014). Over the last 30 years, type 2 diabetes has changed from a relatively mild ailment associated with aging to one of a major cause of premature mortality and morbidity in most countries. The biggest populations of diabetics worldwide are to be found in India (51 million), China (43 million), and the USA (27 million). Nevertheless, there is a worsening global epidemic of diabetes, with almost every country showing a rise in prevalence of diabetes (Centers for Disease Control and Prevention, 2011; Lam and LeRoith, 2012). Several types of drugs such as sulfonylurea, thiazolidinedione, incretin, and dipeptidyl-peptidase 4 inhibitors have been prescribed for treating hyperglycemia in Type 2 diabetic patients. These drugs work by reducing insulin resistance and improving insulin secretion capacity (Kahn *et al.,* 2014). However the adverse side effects and high cost of purchase of these drugs still remains a major problem to people living with diabetes. There is therefore a need to explore all the natural products prescribed by herbalist that are speculated to have anti-diabetic activities, so as to establish a scientific basis for recommending these natural products. This work focused on the use of Ginger and Cinnamon which is widely used spices as hypoglycemic agents. This study could add knowledge about the effects of ginger and cinnamon on diabetes that might be useful in encouraging its possible use in management of diabetes mellitus.

1.6 Justification

The use of herbs to manage diabetes mellitus is crucial, to avoid the undesirable side effects associated with the use of modern day drugs. Ginger and cinnamon are some of the herbs used in the management of diabetes mellitus; they are chosen for this study because they are readily available and used in most households as spices. Although various studies have been published

on the antidiabetic activity of ginger and cinnamon separately, this spiked my interested in finding out how their combination will affect diabetes mellitus.

1.7 Aim and objectives

Aim

This work is aimed at investigating the hypoglycemic and hypolidemic activities of aqueous extract of Ginger (*Zingiber officinale*) and Cinnamon (*Cinnamomum zeylanicum*) and a combination of both on Alloxan induced diabetic rats.

Objectives

The specific objectives of this study are to:

I. Assess the antidiabetic effect of aqueous extract of ginger, cinnamon and their combination on normal and alloxan induced diabetic rats.
II. Assess the effect of withdrawal of treatment with the aqueous extracts of ginger, cinnamon and their combination on alloxan induced diabetic rats.
III. Assess the effects of the aqueous extract of ginger, cinnamon and their combination on Electrolytes (Sodium, potassium, Chloride), Urea and Creatinine.
IV. Assess the effect of aqueous extract of ginger, cinnamon and their combination on the lipid profile of normal and alloxan induced diabetic rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Traditional medicine

Medicinal plants and its constituents have been used for disease management since ancient time. Plants such as *curcumin*, black seed, olive fruits/leaves and dates shows a therapeutic role in disease control via modulation of biological activities (Aldebasi *et al.*, 2013; Rahmani *et al.*, 2014). In Islam, herbs and its constituents have important value in diet and treatment of various diseases and Prophet Mohammed (PBUH) used various herbs including dates and *Nigella sativa* and also recommended various medicinal plants in the diseases cure (Al-Bukhari, 1976). These plants and their constituents show a vital effect in the diseases cure especially with properties of being antioxidant, anti-inflammatory, anti-diabetic and anti-tumour (Arshad *et al.*,2014).

2.2 Diabetes mellitus

Diabetes mellitus is a heterogeneous group of disorders characterized by insulin hypersecretion and/or insensitivity; it is not a single entity but a group of conditions characterized by chronically raised plasma glucose concentration. The relative insulin resistance leads to insufficient action of insulin; a consequence of which is increased blood glucose (hyperglycemia), which in turn leads to other metabolic abnormalities such as increase in ketone bodies as a result of fatty acid breakdown when there is severe insulin insensitivity (Paul *et al.*, 2005).

A variety of factors have been implicated as the causal agents in the development of diabetes. These include, inheritance, infection with a specific bacteria or virus, exposure to food borne chemical toxins which can injure the pancreas, surgical removal of majority (or all) of the pancreas, increase in glucose production (from glycogen degradation) especially at appropriate

times (typical cause of deranged insulin levels), impaired beta-cell function-loss, chronic obesity, increasing age, high fat diet and physical inactivity (Jeloder *et al.,* 2005).

2.2.1 Types of diabetes mellitus

Until recently, diabetes was classified into type 1 and type 2 diabetes, with several other types of diabetes, including gestational diabetes, drug-induced diabetes etc. (Lin and Sun, 2010). Type1 diabetes involves autoimmune destruction of pancreatic islet beta-cells, and renders patients completely dependent on insulin and occurs mainly in children. Type 2 diabetes initially involves insulin resistance, leading to hyperinsulinemia, and is associated with obesity and metabolic syndrome, but eventually progresses to involve pancreatic beta cell dysfunction with an insulin deficit and most diabetics (over 90%) are type 2 diabetics (Kahn *et al.*, 2014). Recently studies showed that diabetes is a group of perhaps hundreds of diseases with different etiologies, all of which involve pancreatic beta cell dysfunction. For example Maturity Onset Diabetes of the Young (MODY) is an autosomal dominant inherited form of diabetes that affects young adults and can be caused by numerous mutations, including mutations of the glucokinase gene and mutations in genes that control beta cell differentiation. MODY does not involve destruction of beta cells in the pancreas or insulin resistance unlike classic type 1 and type 2 diabetes, but consists of malfunctioning beta cells that do not secrete insulin normally in response to increases in blood glucose. Latent autoimmune diabetes of adults (LADA), on the other hand, resembles type1 diabetes very closely, but autoimmune destruction of beta cells is a slower process, taking many years, so that LADA presents in later adulthood. LADA is sometimes mistaken for classic type 2 diabetes, but it does not involve insulin resistance (Gardner and Tai, 2012).

"Mitochondrial diabetes" is another group of diabetic diseases, involving mutations in mitochondrial transfer RNA genes and cause defects in mitochondrial function. Mitochondria are

involved in signaling in the glucose-stimulated increase secretion of insulin by beta cells, so patients with mitochondrial diabetes have defects in this signaling pathway Mitochondrial diabetes also does not involve insulin resistance or pancreatic beta cell destruction, and so cannot be classified as classic type 1 or type 2 diabetes (Lin and Sun, 2010; Sylvia *et al.*, 2014; Steven *et al.*, 2014).

Diabetes is a very complex disease, to add to its complexity it is now clear that some patients who were thought to be classical type 2 diabetes do not have insulin resistance. An emerging concept is that there are thin individuals who are prone to type2 diabetes and there are obese people who are relatively resistant to developing diabetes. This agrees with the idea that diabetes is a heterogeneous group of diseases and that the distinction between type 1 and type 2 diabetes is not as clear as once was thought (Steven *et al*., 2014; Kahn *et al.*, 2014).

All forms of diabetes are characterized with reduced secretion of insulin by beta cells in response to elevations of blood glucose. This may be due to beta cell destruction as in type 1 diabetes and LADA, poor signaling of glucose-stimulated insulin secretion as in mitochondrial diabetes or MODY or poor differentiation of beta cells as in some forms of MODY. Insulin resistance in classic type 2 diabetes progresses only to overt diabetes when insulin hypersecretion exceeds the capacity of the pancreas to secrete insulin, due to toxicity of glucose, lipids and other metabolic products causing pancreatic beta cell death. It may be that type 2 diabetes really is a genetic predisposition to beta cell death in the presence of insulin resistance (Kahn *et al.*, 2014).

2.2.2 Effect of Diabetes mellitus on glucose metabolism and insulin secretion

Insulin is an endocrine hormone secreted in the pancreas by the β-cells of the islets of Langerhans. Its principal function is to aid the passage of glucose across the cellular membrane into the cells. When insulin is deficient or lacking as in the case of diabetes, only a small amount of glucose can cross the cell membrane and used in cellular metabolism. This low rate of transport results in excess accumulation of glucose in the blood (hyperglycemia). As well as urinary excretion of glucose (glucosuria) results when the concentration of blood glucose exceeds the threefold levels for total reabsorption by the kidney (Tharp and Woodman, 2008).

2.2.3 Effect of Diabetes mellitus on lipid profile parameters

Diabetes is a complex disease which impairs carbohydrates and fats metabolisms (O'Keefe and Bell, 2007). Diabetic dyslipidemia is the most typical lipoprotein disorder pattern in diabetes (Solano and Goldberg, 2006). Insulin affects many aspects of mammalian lipid metabolism, it stimulates synthesis of fatty acids in liver,adipose tissue and in the intestine, the insulin has also been reported to increase the cholesterol synthesis (Suryawanshi *et al*., 2006). Low insulin levels are associated with high levels of chylomicrons and very-low-density lipoprotein (VLDL) and lipoprotein lipase deficiency, resulting in hypertriglyceridaemia. Better control of diabetes mellitus improves hyperlipidemia thereby decreasing the risk of coronary heart disease (Manley *et al*., 2000, Holman, 2001 and Sacks *et al.,* 2002).

Insulin resistance causes an increase in VLDL levels and consequently dyslipidemia (Rader & Hovingh, 2014). Type 2 diabetes is associated with a cluster of interrelated plasma lipid and lipoprotein abnormalities, including reduced HDL cholesterol, a predominance of small dense LDL particles, and elevated triglycerides (American Diabetes Association, 2003).make sure its in ref list

Diabetes mellitus is known to be associated with impaired insulin action, this is results in defective suppression of intracellular hydrolysis of triglycerides which in turn results in the release of free fatty acids into circulation (Ragheb and Medhat, 2011). The increased influx of free fatty acids from adipose tissue to the liver promotes triglyceride synthesis and the assembly and secretion of large very low density lipoprotein; this result in elevated plasma very low density lipoprotein levels, increase hepatic glucose production, decrease insulin clearance and induce peripheral insulin resistance (Vijayaraghavan, 2010).

2.2.4 Oxidative stress in diabetes

Hyperglycemia maybe a cause of oxidative stress in diabetes mellitus, it stimulates free radical production and weakens the defense system of the body thereby causing a condition of imbalance in reactive oxygen species resulting in oxidative stress (Pandey *et al.*, 2010). For normal metabolic activities, certain amount of oxidative stress is necessary since reactive oxygen species play various regulatory roles in cells (Gomes *et al.*, 2012). For instance, they are produced by neutrophils and macrophages during the process of respiratory burst in order to eliminate antigens (Freitas *et al.*, 2010). They also serve as stimulating signals of several genes (Gomes *et al.*, 2012).

However uncontrolled production of ROS is harmful. Metabolic abnormalities of diabetes cause mitochondrial superoxide overproduction in endothelial cells of both large and small vessels, as well as in the myocardium (Giacco and Brownlee, 2010; Patel *et al.*, 2013). Oxidative stress acts as mediator to inhibit insulin gene expression, suppress insulin gene transcription, and induce alteration of glucose-stimulated insulin secretion by damage to mitochondrial DNA and membrane proteins and by down regulation of glyceraldehyde-3-phosphate dehydrogenase, which results in loss of intracellular and mitochondrial ATP (Chon *et al.*, 2014).

2.3 Alloxan

Alloxan is a compound which is toxic by selectively destroying insulin-producing cells in the pancreas (β-cells) of many animal species. This causes an insulin-dependent diabetes mellitus also known as "Alloxan Diabetes" in these animals, with characteristics similar to type 1diabetes in humans. Alloxan is taken up by GLUT2 glucose transporter to the beta cells where it preferentially accumulates and selectively intoxicates the insulin producing pancreatic beta cells. In the presence of intracellular thiols, it generates reactive oxygen species (ROS) which initiate toxicity by its redox reaction (Lenzen, 2008).

2.3.1 Mechanism of action of alloxan

Alloxan exerts its pathological effects in two ways: it selectively inhibits glucose-induced insulin secretion via specific inhibition of glucokinase, the glucose sensor of the beta cell, thereby causing a state of insulin-dependent diabetes through its ability to induce ROS formation, resulting in the selective necrosis of beta cells. These two effects are specific chemical properties of alloxan, what is common to both is their selective cellular uptake and accumulation of alloxan by the beta cell. (Lenzen, 2008)

2.3.2 Effect of Alloxan on beta cells

Alloxan been an unstable chemical compound (Lenzen and Munday, 1991) with a molecular shape similar to glucose (Gorus *et al.,* 1982) is also hydrophilic like glucose and does not penetrate the lipid bilayer of the plasma membrane. Because of its glucomimetic ability the GLUT2 glucose transporter in the beta cell plasma membrane accepts to transport it into the cytosol. (Weaver *et al.,* 1978). According to Elsner *et al.* (2002) Alloxan does not inhibit the function of the transporter and can therefore selectively enter beta cells in an unrestricted manner

(Boquist *et al.,* 1983; Malaisse *et al.,* 2001). It is therefore not toxic to insulin-producing cells that do not express this transporter (Hammarström *et al.,* 1967; Bloch *et al.,* 2000). Alloxan has a short half-life (Patterson *et al.,* 1949) therefore in aqueous solution it spontaneously decomposes into non-diabetogenic alloxanic acid within minutes (Lenzen and Munday, 1991). Because of this, it must be taken up and accumulated quickly in the beta cell (Hammarström *et al.,*1967) and is therefore ineffective when blood flow to the pancreas is interrupted for the first few minutes after alloxan injection (Gomori and Goldner, 1945;Bailey *et al.,* 1950).

2.3.3 Glucokinase inhibition by alloxan

Selective inhibition of glucose-induced insulin secretion is the major pathophysiological effect of the thiol group reactivity of alloxan (Hara *et al.,* 1986; Meglasson *et al.,* 1986;. Lenzen *et al.,* 1987). Alloxan has a central 5-carbonyl group that reacts very avidly with thiol groups. Glucokinase (hexokinase IV) is the most sensitive thiol enzyme in the beta cell (Tiedge *et al.,* 2000), with a half maximal inhibitory concentration in the 1–10 μmol/l range. At higher concentrations, alloxan can inhibit many important enzymes, as well as other proteins and cellular functions (Konrad and Kudlow, 2002). Inhibition of glucokinase reduces glucose oxidation and ATP generation (Gunnarsson and Hellerström, 1973) thereby suppressing the ATP signal that triggers insulin secretion. Inhibition of glucokinase is achieved within 1 min of exposure to alloxan. The inhibition of glucose-induced insulin secretion is preceded by a very transient (1–2 min) stimulation of insulin secretion immediately after exposure to alloxan (Weaver *et al.,* 1978). This effect can be explained by an initial reduction of ATP consumption resulting from the blockade of glucose phosphorylation by glucokinase (Lenzen and Panten, 1988) which produces a transient increase in ATP in the beta cell and triggers a transient release of insulin.

The inhibition of insulin secretion after exposure to alloxan (Weaver *et al.*, 1978; Ishibashi *et al.*,1978 ; Miwa *et al.*,1986; Lenzen and Panten, 1988) is restricted to that induced by glucose and its epimer, mannose, both of which induce insulin secretion through interaction with glucokinase (Lenzen and Panten, 1988). Insulin biosynthesis is also inhibited by alloxan (Jain and Logothetopoulos, 1976; Niki *et al.*, 1976), most likely through the same mechanism. The insulin secretory response to other nutrient secretagogues, such as 2-ketoisocaproic acid and leucine, or non-nutrient secretagogues, such as the sulfonylurea drug tolbutamide, remains intact initially because it is not mediated via glucokinase (Lenzen *et al.*,1988), but is lost after a delay of up to 1 h (Borg ,1981) as a consequence of the gradual deterioration of beta cell function.

2.4 Ginger

Ginger (*Zingiber officinale*) is one of the most widely spices used in the world. It originated from South-East Asia and then became widespread to many parts of the world. It has been cultivated for centuries as a spice and condiment to add flavor to Indian food (Park and Pizzuto, 2002). Besides its extensive use as a spice, the rhizome of ginger has also been used in traditional herbal medicine. The health-promoting perspective of ginger is often attributed to its rich phytochemistry (Shukla and Singh, 2007).

Fig1: Ginger rhizome

2.4.1 Plant description

Ginger (*Zingiber Officinale*) is a tropical perennial plant with narrow, bright green grass-like leaves and yellowish green flowers with purple markings and an aromatic underground stem, called a rhizome.(Chrubasik *et al.*,2005; Al-Amin *et al.*, 2006; NSRC,2009;). The plant was named by an English botanist William Roscue in 1807 (El-Bushuty and Shanshan, 2012). Ginger plants have been used for cooking and treating a host of ailments throughout Asia, especially in India and China, for over 5000 years. It can be consumed as a fresh or dried root and is often prepared in teas, soft drinks, and breads. According to European Pharmacopoeia 2011, ginger consists of the whole or cut rhizome of *Zingiber officinale* Roscoe with the cork removed, either completely or otherwise (Ovesen, 2012).

2.4.2 Uses of ginger

The rhizome (the roots) can be peeled and is used as a spice in cooking and baking, and has long been used in the traditional Chinese and Indian medicine, or so-called alternative or complementary medicine, with limited scientific evidence of its benefits or safety. (NSRC, 2009) It can be used fresh, dried, powdered, as a tea, juice or oil. The oil can be taken orally or applied to the skin.

The major pharmacological activity of ginger maybe due to the presence of gingerols and shogaol. It is used to treat nausea due to any of the following; motion sickness, morning sickness, general anesthesia and chemotherapy, it also has anti-inflammatory and antioxidant properties which help to relieve various inflammatory disorders like gout, osteoarithritis and rheumatoid arithritis (Haniadka *et al.*,2013). It also has analgesic and hypoglycemic actions (Ojewwole, 2006). Experimental studies demonstrated that ginger in a crude or standardized extract has cardiotonic, antiplatelets, antiemetic, antimicrobial, antifungal actions

andantidiabetic, antidyslipidemia; antiobesity, anticancer activity (Singh *et al.*, 2010). It also has hypotensive, vasodilator effects and verapamil like effects on the heart (Elkhishin and Awwad, 2009). It is also reported to be protective against tumour formation, lipid peroxidation , also it has anti-pyretic and anti-inflammatory effect which may be useful in relieving musculoskeletal and osteo-arthritic pain.(Chrubasik *et al.*,2005)

2.4.3 Phytochemistry of ginger

Ginger plants contain volatile oil 1-4%. According to the European Pharmacopeias, more than 100 different compounds have been identified in ginger, most of them terpenoids and sesquiterpenoids (zingiberine, bisabolene, and zingibrol) and small amounts of monoterpenoids (camphene, cineole, geraniol, curcumene, and borneol). The pungent principles, the gingerols (4-7.5%), are a homologous series of phenols. The principal one of these is 6- gingerol. Gingerols with other chain-lengths *e.g*. 8-gingerol and 10-gingerol, are present in smaller amounts. During drying and storage, gingerols are partly dehydrated to the corresponding shogaols which may undergo further reduction to form paradols. Other constituents are starch up to 50%, lipids 6-8%, proteins, and inorganic compounds (Ovesen, 2012).

The crude ginger plant material has many other sulfonated compounds and shogasulfonic acids. These phenolic shogaols are much more pungent than gingerols, and are mainly found in the semi-dried ginger plant. Shogaols are rarely found in fresh ginger, as they are major degradation products of gingerols (gingerols are thermally labile, thus the shogaols will be released when the ginger is heated and dried). Zingerone is also a degradation product and this will cause the ginger product to smell bad as it ages.(Chrubasik *et al.*, 2005).

The gingerols and the shagaols are volatile and occur in any of the following forms n-gingerol (n=6, 8, 12), and n- shogaol (n=6, 8, 12). Other volatile compounds include

The volatile ginger components consist of n-gingerol (n=6, 8, 12), n-shogaol (n=6, 8, 12), [6]-paradol, [6]-methylgingerdiol, [6]-methylgingerdiacetate,gingerdiones,[6]-gingesulfonicacid,[6]-hydroxyshogaol,and hexahydrocurcumin (Bhargava *et al*, 2012, Ahui *et al*. 2013, Rahmani *et al*, 2014). Ginger also contains other volatile compounds such as α-pinene, camphene, 6-methyl-5-hepten-2-one, myrcene, α- and β-phellandrene, limonene (Mesomo *et al*, 2013).

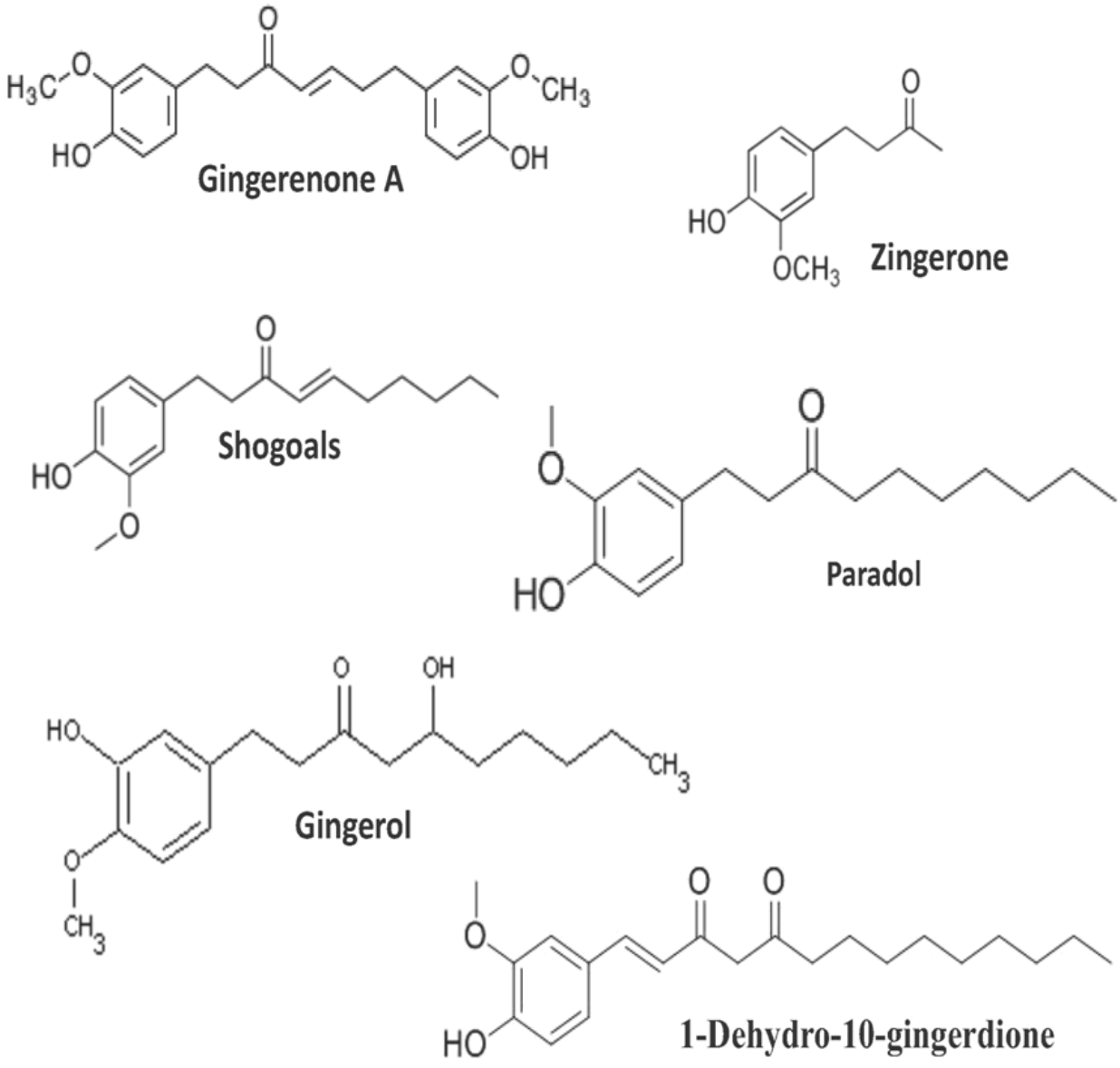

Fig 2: Some active constituents of ginger.

2.4.4 Effects of Ginger in Diabetes Mellitus

Studies have shown that ginger has hypoglycemic effects and it decreases the complications of diabetes mellitus, in these studies ginger extract was used in various doses for different periods and through different routes of administration in both types of diabetes mellitus, it was observed from these studies that administration of ginger extract significantly reduced blood glucose in animals induced with type1 diabetes mellitus. (Al-Amin *et al.,* 2006; Elshater *et al., 2009;* Shanmugam *et al.,* Jafari *et al.,*2011; Morakinyo *et al.,* 2011). Hypoglycemic effects of ginger are dose dependent. An acute study indicated that administration of 4 and 8mg/Kg of ginger intraperitoneally to rats after 30 minutes of diabetes induction showed pronounced hypoglycemic effect after 2 hours (Abdulrazaq *et al.,* 2011; Kalejaiye *et al.,* 2002). A component of ginger, 6-gingerol exhibited hypoglycemic effect when administered to diabetic mice. Ginger also has hypoglycemic effects on normal rats, mice, rabbits and broiler chick (Khulood, 2014)

Clinical trials revealed that ginger supplementation lowered blood glucose in patients with type 2 diabetes mellitus when administered for a long period in addition to improving insulin senstivity and lipid profile (Andallu *et al.,* 2003).

Prolonged treatment with ginger improves blood glucose levels as well as decrease total cholesterol and triglyceride; it also increases insulin and prevents body weight loss in type1 diabetic animals (Al-Amin *et al.,* 2006). Another study reported that ginger had a protective effect on development of various parameters of metabolic syndrome in high-fat diet (HFD) fed rats. High fat diet is a predisposing condition to type2 diabetes. Treatment of these animals with an ethanolic extract of ginger at dose of 100, 200, and 400mg/Kg for six weeks showed a significant reduction in free fatty acids, high serum glucose, high triglycerides, high LDL

cholesterol, high total cholesterol, high insulin, high body weight and high phospholipids induced by the high fat diet(Nammi *et al.,*2009)

Madkor *et al.,* (2010) showed in a study that oral administration of ginger powder at a dose of 200mg/Kg to Streptozotocin induced diabetic rats alleviated signs of metabolic syndrome i.e. decrease blood glucose, total lipids and increased total antioxidant level.

Better glucose tolerance and enhanced insulin concentration were observed in ginger treated type2 animals induced by a combination of high fat diet and Streptozotocin. The major pungent component of ginger (6-gingerol) significantly reduced fasting blood glucose and improved glucose tolerance. It also lowered plasma triglyceride, total cholesterol, LDL and free fatty acids when administered at a dose of 100mg/Kg body weight (Singh *et al.,* 2009).

Ethanolic extract of ginger when administered to a high fat diet fed rats at a dose of 400mg/kg effectively reduced triglyceride and cholesterol level in the liver. Molecular studies showed that mRNA and protein expression of LDL receptor is increased while at the same time HMG-CoA(3-hydroxy-3-methylglutaryl coenzyme A) reductase activity was downregulated. This suggests that the lipid homeostasis effect of ginger was partially due to a decrease in cholesterol biosynthesis and in enhanced hepatic uptake of circulating LDL cholesterol (Nammi *et al.,* 2010).

It was reported by Shanmugam *et al.,* (2011) that feeding a group of normal rats and Streptozotocin induced diabetic rats with a diet containing 1% and 2% ginger respectively for 30 days significantly reduced the blood glucose level in the diabetic rats while it remained unaffected in the normal rats. Feeding diabetic rats with ginger decreases the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP*x*) and glutathione reductase (GR) in the hepatic and renal tissues thereby causing a decrease of malondialdehyde

(MDA) and an increment in glutathione (GSH) level, this therefore helps to oxidative damage to the liver and the kidneys. Treatment of diabetic rats with ginger showed recovery from the degeneration of the liver and the sinusoids, it also improved alterations of the kidneys.

In another study feeding Ethanolic ginger extract to Streptozotocin induced diabetic rats reduced blood glucose level, glycogen, pyruvate, and total protein in their kidney tissues. It was further discovered that the decreased activities of glucose 6-phosphate dehydrogenase (G6PD), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), and glutamate dehydrogenase (GDH) in diabetic rats were reversed after treatment with 200mg/kg of ginger for 30 days. Histological examination showed ginger treatment appeared to regenerate tubules, restore glomeruli, and reduce fatty infiltration (Shanmugam *et al.,* 2009).

Intraperitoneal injection of aqueous ginger extract to Streptozotocin diabetic rats for 7 weeks lowered their serum glucose and urine protein to the same level as the normal group (Yiming *et al.,* 2014).

Limited clinical trials have been conducted to demonstrate the beneficial effect of ginger in human subjects. However Andallu *et al.,* (2003) stated that consuming 3g of dried ginger powder divided in 30 portions for 30 days showed a significant reduction in total cholesterol, LDL, VLDL, triglyceride and blood glucose in diabetic patients.

2.4.5 Possible Mode of Action of Ginger on Glycaemic Control

1. Inhibition of some Enzymes in Carbohydrate digestion.

α-amylase and α-glucosidase are key enzymes associated with carbohydrate digestion in a state of hyperglyceamia and type diabetes, an invitro enzyme study conducted using water, 70%methanol-water, hexane, and ethyl-acetate extract of ginger showed that the ethyl acetate extract had the highest inhibitory activity against α-glucosidase and α- amylase with an IC50

values of 180 mg/mL and 980 mg/mL, respectively. The action of ginger against these enzymes was found to be correlated to the phenolic contents of gingerol and shagoal in the extract (Priya Rani *et al.,* 2011)

2. Increases in Insulin Release and Sensitivity.

A phenolic component of ginger extract [6]-gingerol had a protective effect on the pancreatic *β*-cell of arsenic induced type 2 diabetic rats thereby restoring the plasma insulin level. Although the mechanism is not yet fully understood, it is believed that the ginger interacts with 5-HT3 receptor. Gingerols and shagaols were found to act by binding to a modulatory site different from the serotonin binding site on the 5-HT3 receptor (Priya Rani *et al.,* 2011). Ginger plays an important role in maintaining homeostasis of blood glucose in diabetes patients by promoting glucose clearance in insulin responsive tissues. In vitro studies showed that gingerol encourages glucose uptake in cultured rat skeletal muscle cells and adipocytes (Yiming *et al.*, 2012).

3. Improves Lipid Profiles.

Insulin resistance in peripheral tissues in diabetic patients is known to be closely associated with accumulation of circulating lipids and tissue lipid (McGarry, 2002). The first rate limiting step in glucose metabolism is its entry into the peripheral tissues is inhibited by excessive fatty acid oxidation. Many studies showed ginger to have antihyperlipidaemic activity hence increasing insulin sensitivity.

Clinical trials have showed promising results of improved lipid profile in diabetic patients (Andallu *et al.*, 2003). When ginger is combined with other herbs, some significant physiological changes such as reduced skin thickness, reduced body weight, reduced waist/hip circumference were seen in addition to reduced serum cholesterol and triglyceride in diabetic hyperlipidemic patients (Kamal and Aleem, 2009).

2.4.6 Protective effect of Ginger against diabetic complications

In diabetic patients, glucose can be converted to sorbitol and fructose by the enzyme aldose reductase, accumulation of these compounds can lead to the rapid development of cataract in the eyes (Fuhrman *et al.,* 2000). Some compounds found in ginger such as 2-(4-Hydroxy- 3-methoxyphenyl) ethanol and 2-(4-hydroxy-3-methoxyphenyl) ethanoic acid inhibited aldose reductase in vitro, they also suppressed sorbitol accumulation in human erythrocytes and lens galactitol accumulation in the galactose-fed cataract rat model (Kato *et al.,* 2006).

2.4.7 Toxicological Studies of Ginger

Toxicological studies on rats determined the LD_{50} (oral) of aqueous extract as 11.75g/Kg and that of methanolic extract as 10.25g/Kg (Shalaby and Hamowieh, 2010). When administered intraperitoneally the LD_{50} was 1551±75mg/Kg (Ojewwole, 2006).

Rong *et al.,* (2009) conducted a 35 day toxicity test on rats to determine the effect of prolonged ginger use. The result showed that oral administration of up to 2g/Kg of ginger caused no abnormality or abnormal changes in the general condition or heamatological parameters for both male and female rats. In a different study it was shown that intraperitoneal administration of ginger at a dose of 500 mg/kg for 28 days showed slight toxicity in the heamatological parameters, liver enzymes and systemic toxicity in liver and lung tissues (Al-naqeeb *et al.*,2003).

2.5 Cinnamon

Cinnamon (*Cinnamonum zeylanicum*) is a small evergreen tree, 10-15 meters (32.8-49.2 feet) tall, belonging to the family Lauraceae, native to Sri Lanka and South India, but is currently grown all over the world. The flowers are green in color with a distinct odour, they are also arranged in panicles. The fruit is a purple one-centimeter berry containing a single seed. Its

flavour is due to an aromatic essential oil which makes up 0.5 to 1% of its composition (Vaibhavi *et al.,* 2010). Cinnamon is mainly used in the aroma and essence industries due to its fragrance, which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products (Rao and Gan, 2014).

It is widely used as a spice and flavoring agent. It is used in chewing gum making to add flavor to the gum due to its ability to remove bad breath thereby keeping the mouth refreshed (Jakhetia *et al.,* 2010). It is used as an anticoagulant to prevent bleeding (Wondrak *et al.,* 2010). It improves colon health thus reducing the risk of colon cancer (Hossein *et al.,* 2013). It was also found to increase blood circulation in the uterus and promotes tissue regeneration (Rao and Gan, 2014). It was also shown to have antimicrobial (Chang *et al.,* 2001), antifungal (Wang *et al.,* 2005), antioxidant (Kim *et al.,* 1993) and antidiabetic (Kim *et al.,* 2006) activities.

Fig 3: Cinnamon bark

2.5.1 Phytochemistry of cinnamon

Table1 below shows the phytochemical constituents of different extracts of *Cinnamonum zeylanicum* (Pandey *et al.,* (2014).

The chemistry of these compounds showed a variety of resinous compounds including cinnamaldehyde, cinnamate, cinnamic acid and other essential oils (Senanayake *et al.,* 1978). It was also reported by Singh *et al.,* (2007) that cinnamaldehyde is responsible for the sweet fragrance and spicy taste of cinnamon, this occurs to the cinnamaldehyde's ability to absorp oxygen. Cinnamon darkens in colour with age (Singh *et al.,* 2007) this is as a result of improvement in resinous compounds.

Table 1: Phytochemical screening of Cinnamon

phytoconstituents	Cold water extract	Hot water extract	Warm water extract	Ethanol extract	Methanol extract	Acetone extract
Carbohydrates	+	+	+	–	–	–
Steroids	+	+	+	+	+	+
Proteins	–	–	–	–	–	–
Glycosides	–	–	–	–	–	–
Alkaloids	+	+	+	+	+	+
Flavonoids	+	–	–	+	–	–
Saponins	+	+	+	+	+	+
Tannins & phenols	–	–	–	+	+	+

Adapted from Pandey *et al.*,(2014)

2.5.2 Anti-diabetic effect of cinnamon

Insulin potentiating factor (IPF) is a compound isolated from cinnamon which is shown to have anti-diabetic activity (Khan *et al.,* 1990). Studies have shown that cinnamon lowers blood glucose as well as cholesterol (Blevins *et al.,* 2007). Broadhurst *et al.,* (2000) carried out a study comparing antidiabetic effects of many spices; it was observed that aqueous extract of cinnamon has 20 times more antidiabetic activity than other spices. Polyphenol type A polymers from cinnamon were isolated and characterized by Anderson *et al.,* (2003) and these polymers were found to have insulin-like activities. Furthermore a compound named naphthalenemethyl ester was discovered in hydroxycinnamic acid derivatives, this compound is also shown to have antidiabetic effects, and this further confirms that cinnamon is antidiabetic (Kim *et al.,* 2006). Other studies showed that administration of cinnamon in doses of 5, 10, and 20mg/kg was found to help glycemic control and enhance insulin secretion in diabetic patients. It is possible that reduction of oxidative stress and pro-inflammatory in the pancreas may protect the β cells of the pancreas (Lee *et al.,* 2013)

The aqueous extract of cinnamon significantly decreased the absorption of alanine in the rat intestine. Alanine plays a vital role in gluconeogenesis, it is converted to pyruvate in the liver, and is utilized as a substrate for gluconeogenesis (Kreydiyyeh *et al.,* 2000). Cinnamon extract significantly improved glucose infusion rates in rats when compared to the controls. The insulin-stimulated insulin receptor (IR) β and IR substrate-1 (IRS1) tyrosine phosphorylation levels and IRS1/phospho-inositide 3-kinase (PI3K) in skeletal muscle of chow diet-fed rats are enhanced by cinnamon extract (Qin *et al.,* 2003)

Table 2: Some clinical trials that used cinnamon extracts for glycemic control

Condition /disease	Study type	Author /year	No. of patients	Statistically significant	Comments	Dose
Post parandial blood glucose-attenuating satiety enhancing effect	On healthy human volunteers	Mettler *et al.*,2009	27	Yes	Reducing the blood glucose and enhance satiety post parandial	4g for once in the meal
HbA1c in patients with type 2 diabetes	A randomized controlled trial	Crawford,2009	109	Yes	Taking cinnamon could be useful for lowering serum HbA1C in type 2 diabetics with HbA1C(>7.0) in addition to usual care	1g/day for 90 days
People with impaired fasting glucose that are overweight or obese	A double blind placebo-controlled trial	Rousel *et al.*,2009	22	Yes	The inclusion of water soluble cinnamon compounds in the diet could reduce risk factors associated with diabetes and cardiovascular disease	250mg two times a day for 12 weeks
Gastric emptying, satiety,and post parandial blood glucose,insulin, glucose dependent insulinotropic polypeptide, glucagon-like peptide 1 and ghrelin concentrations	A crossover trial on healthy volunteer	Hlebowicz *et al.,* 2009	15	Yes	Cinnamon have got the potential to modulate gastric emptying, satiety, and postparandial blood glucose, insulin, glucose-dependent insulinotropic polypeptide, glucagon-like peptide 1, and ghrelin concentrations	1-3g
Nasal allergy/allergic rhinitis	A pilot randomized double-blind placebo controlled trial randomly assigned	Coren *et al.*,2008	>20	Yes	Cinnamon has got the potential clinical utility in patients with allergic rhinitis	Not specified
Urinary oxalate excretion, plasma lipids	Crossover study in healthy volunteers	tang *et al.*,2008	11	No	Cinnamon has no effect on urinary oxalate excretion and plasma lipid profile	Consumed in the diet

Condition /disease	Study type	Author /year	No. of patients	Statistically significant	Comments	Dose
Invivo glucose tolerance	A randomized healthy male volunteers crossover design	Solomom and Lannin, 2007	7	yes	Cinnamon spice supplementation may be important to invivo glycemic control and insulin sensitivity in humans, and not only are its effect immediate , they also appear to be sustained for 12 hours	5g/day
Type 2 diabetes mellitus	Placebo controlled trial	Suppapitipom *et al.,* 2006	79	No	No significant effect was observed in type 2 diabetes mellitus	1.5g/day for 12 weeks
Plasma glucose, HbA and serum lipids in diabetes mellitus type 2	Double blind randomized placebo-controlled trial	Mang *et al.,* 2006	79	Yes	Cinnamon extract seems to have a moderat effect in reducing fasting plasma glucose concentration in diabetic patients with poor glycemic control	3g/day for 4 months

Adapted from Vaibhavi *et al.,* (2010)

The decreased glucose infusion rate in HFD-fed rats (60% of normal controls) was improved by Cinnamon extract to the level of controls, and the improving effects of the extract on the glucose infusion rates of HFD-fed rats were blocked by *N*-monomethyl-L-arginine (an inhibitor of nitric oxide, NO). The decreased muscular insulin-stimulated IRβ and IRS1 tyrosine phosphorylation levels and IRS1 associated with PI3K in HFD-fed rats are also improved significantly by the extract. These data suggest that Cinnamon extract prevents the development of insulin resistance, at least in part by enhancing insulin signaling and possibly via the NO pathway in skeletal muscle (Wang *et al.,*2007) It was shown that cinnamon extract improved glucose utilization in high fructose diet (HFD) fed rats(Qin *et al.*, 2004) .

Studies showed that Cinnamon extract increases glucose uptake and GLUT4 expression in 3T3-L1 adipose cells, GLUT4 is a glucose transporter which facilitates the transport of glucose across the plasma membrane into the skeletal muscle and adipose cells. It was also observed that

aqueous extract of cinnamon (Cinnulin PF®) reduced plasma insulin, blood glucose and CD36, which is a soluble cluster of differentiation 36. This CD36 is reported as a novel marker of insulin resistance. Retinol-binding protein 4 (RBP4): a novel adipokine which promotes insulin resistance was shown to be inhibited by cinnamon extract (Handberg *et al.*,2006)

Cinnamon extract consumption also appears to regulate glucose uptake-related genes, such as Glut1, Glut4, glycogen synthesis 1, and glycogen synthase kinase 3β mRNA expression in adipose tissue (Qin *et al.,* 2010).

2.5.3 Anti-inflammatory effect of cinnamon

There is growing evidence of a strong link between type2 diabetes mellitus and systemic inflammation, the high level of elevated inflammatory cytokines may contribute to insulin resistance(Qin *et al.,* 2010) tristetraprolin is an anti-inflammatory protein and a potential therapeutic target for the treatment of inflammation-related diseases. The expression of this protein is found to be reduced in adipose tissue of obese individuals with metabolic syndrome. This protein Tristetraprolin is believed to offer partial protection against the development of insulin resistance and diabetes (Duncan *et al.,* 2003; Cao *et al.,* 2008). Studies showed that cinnamon extract rapidly rapidly induces the expression of tristetraprolin mRNA levels in 3T3-L1 adipocytes. (Cao *et al.,* 2008)

The pro-inflammatory cytokine, tumor necrosis factor (TNF)-α, which is a link among obesity, insulin resistance, and metabolic syndrome, stimulates the overproduction of intestinal apolipoprotein B48 (apoB48)- containing lipoproteins (Bouchard *et al.,* 2007) *In vivo* oral treatment with Cinnulin PF inhibits the postprandial overproduction of apoB48-containing lipoproteins and serum triglyceride levels in rats and hamsters. In *in-vitro* 35 S-labeling studies Cinnamon extract inhibited the over-secretion of apoB48 induced by TNF-α-treated enterocytes

into the medium. Cinnamon extract treatment decreases the mRNA expression of the inflammatory factors [interleukin (IL)1β, IL6, and TNF-α]; improves the mRNA expression of IR, IRS1, IRS2, PI3K, and Akt1; inhibits CD36, microsomal triglyceride transfer protein (MTP), and phosphatase and tensin homolog; and enhances impaired sterol regulatory element-binding protein (SREBP)-1c expression in TNF-α- treated enterocytes (Qin *et al.*, 2007) This study suggested that Cinnamon extract helps prevent the elevation of circulating triglyceride-rich lipoproteins with significant effects on intestinal insulin resistance. Accumulating evidence indicates that dyslipidemia is associated with insulin-resistant states resulting from the overproduction of both intestinal and hepatic triglyceride-rich lipoproteins and the delay of their hepatic clearance. Cinnamon extract reduces inflammation-related dyslipidemia and decreases risk factors associated with cardiovascular diseases (Qin *et al.*, 2010)

2.5.4 Anti-cancer effect of cinnamon

Vascular endothelial growth factor is a mitogenic and angiogenic factor involved in tumor progression, in collateral vessel formation in ischemic tissues, and in inflammation, as well as in the development of diabetic retinopathy (Ferrara, 2004) It is also a key mediator of adipogenesis in obesity and insulin resistance (Nishimura *et al.*, 2007) Moreover it is one of the most critical factors that induce angiogenesis and has thus become an attractive target for anti-angiogenesis treatment. However, most current anti-VEGF agents often cause side effects and therefore cannot be recommended for long-term use. Identification of naturally occurring VEGF inhibitors derived from foods would be one alternative approach to control with an advantage of anticipated safety. Cinnamon extract inhibits VEGF-induced endothelial cell proliferation, migration, and tube formation *in vitro*, sprouts formation from aortic ring *in-vitro*, and tumor-induced blood vessel formation *in vivo* (Lu *et al.*, 2010) While cinnamaldehyde, a component

associated with the aroma of Cinnamon, has little effect on VEGF receptor (VEGFR) kinase activity, high-performance liquid chromatography-purified components of Cinnamon extract, procyanidin type A trimer (MW 864) and a tetramer (MW 1152), inhibit the kinase activity of purified VEGFR and VEGFR signaling pathways. These data suggest that procyanidin oligomers are active components in Cinnamon that inhibit angiogenesis. Taken together, this study revealed novel activity in cinnamon and identified a natural inhibitor of VEGF signaling that could potentially be useful in cancer prevention and/or treatment (Lu *et al.,* 2010).

2.5.5 Lipid-Lowering Effects of cinnamon

The administration of cinnamon to mice improved the lipid profile, whereby the high density lipoprotein (HDL) cholesterol levels decreased, and plasma triglycerides were reduced (Kim *et al.,* 2006). Another study by Rahman *et al.,* (2013) found a reduction in the total cholesterol, triglycerides, and low-density lipoproteins in rats administered *Cinnamomum cassia* powder (15%) for 35 days. Additionally, cinnamon oils reduced the cholesterol levels in broiler chickens (Ciftci *et al.,* 2010). A study by Khan *et al.,* (2003) reported that the administration of cinnamon at 1, 3, and 6 g doses per day caused a reduction in serum glucose, triglyceride, total cholesterol, and LDL cholesterol levels in humans.

2.5.6 Effect of cinnamon on cardiovascular diseases

One of the active components isolated from *C. cassia* named 2-methoxycinnamaldehyde (2-xsMCA) decreases the expression of vascular cell adhesion molecule-1 (VCAM- 1) in TNFα-activated endothelial cells, suggesting that ischemia/reperfusion (I/R) injury is ameliorated due to the induction of hemeoxygenase- (HO-) 1 (Hwa *et al.,* 2011). A recent study reported the potential effects of two compounds, cinnamic aldehyde and cinnamic acid, isolated from *C.*

cassia against myocardial ischemia (Song *et al.,* 2013), indicating that cinnamon also has the potential to be used to treat cardiovascular diseases. Several studies have reported the protective effects of cinnamaldehyde on the cardiovascular system. Cinnamophilin is one of the important lignans isolated from *C. philippinensis* and has been confirmed to have thromboxane A2 (TXA2) receptor blocking activity in rats as well as in guinea pigs (Yu *et al.,* 1994). Cinnamophilin acts as a potential thromboxane synthase inhibitor and TXA2 receptor antagonist and may be helpful when incorporated in the treatment of diseases involving TXA2 disorders (Yu *et al.,* 1994), such as platelet aggregation (Jurasz *et al.,* 2004) and cancers (Nie *et al.,* 2004). Cinnamophilin mainly inhibits thromboxane receptor-mediated vascular smooth muscle cell proliferation and may have the potential for use in the prevention of vascular diseases and atherosclerosis (Ko *et al.,* 1995). Cinnamaldehyde produces hypotensive effects, which are possibly mainly due to peripheral vasodilatation in anesthetized dogs and guinea pigs (Harada and Yano, 1975). A recent study showed that cinnamaldehyde expands rat vascular smooth muscle in an endothelium in dependent manner. The ability of cinnamaldehyde in vasodilatory function may be because it impedes both Ca2+ influx and Ca2+ release (Xue *et al.,* 2011) Cinnamaldehyde averts the progress of hypertension in types 1 and 2 diabetes by abridging vascular contractility, in addition to its insulinotropic effect in insulin deficiency (El-Bassossy *et al.,* 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment

Gallenhamp vacuum oven (OC 4772C) from Island Scientific Limited, BUCK Scientific Absorption Spectrophotometer (210VGP), Accucheck active glucometer from Roche Germany, Thermo Sorvall legend XT bench Centrifuge, weighing balance, cheese cloth, vacuum pump from Welch.

3.1.2 Reagents

Kits for the assay of serum total cholesterol, HDL-cholesterol, triglycerides and chloride were obtained from Randox (UK) diagnostic while potassium, sodium, urea and creatinine assay kits were obtained from Teco diagnostic (USA).

3.1.3 Experimental Animals

The laboratory animals were purchased from National Veterinary Research Institute, Vom. They were then transported to Bayero University Kano. The research was conducted according to Bayero University Kano protocols on the use of animals in research.

3.1.4 Collection and preparation of plant material

Fresh *Zingiber officinale* (ginger rhizome) and dried *Cinnamonum zeylanicum* (cinnamon bark) were bought from ‘Yan Kaba market. They were respectively identified by a botanist at the Biological Science Department of Bayero University Kano, Nigeria with accession number

BUKHAN 0368 and 369. The fresh ginger rhizomes was ground and kept at room temperature in a sealed labeled plastic container, also the cinnamon bark was pulverized to powder and kept at room temperature in a sealed labeled container. Both plant materials were then used immediately for extraction.

3.2. Extraction of plants materials

Five hundred grams (500g) each of ginger and cinnamon were ground and pulverized respectively, 1litre of distilled water was used to soak each of them separately, it was stirred properly and left to stand for 24 hours. After 24 hours the mixtures were then filtered separately using fine cheese cloth of 44x36 threads per inch twice and the filtered using a vacuum pump otherwise known as suction machine to obtain the aqueous extracts. The extract was then stored at 25°C in a container labeled aqueous ginger extract.

3.2.1 Determination of concentration of the extracts

The concentration of the clear debris free filtrate of the sample was determined using Total Dissolved Solute method (TDS). Four petri-dishes were weighed separately and the initial weight noted, ten milliliter of both ginger and cinnamon extracts were taken and placed into two petri-dishes each; the petri-dishes were properly labeled as ginger and cinnamon. The petri-dishes were then placed in an oven at 80°C for 1 hour to completely dry, they were then brought out and cooled. The final weight of both petri-dishes was taken. The difference in the weight was used to determine the amount of the extracts in 10ml of distilled water. On this basis the concentration of the ginger extract was found to be 19 mg/ml while that of cinnamon was 20.5 mg/ml.

3.3 Experimental design

A total of 40 albino rats were used for the study. They were placed in eight groups of 5 rats each.

Group I: Control

Group II: Diabetes was not induced, ginger extract given at a dose of 200mg/kg

Group III: Diabetes was not induced, cinnamon extract given at a dose of 150mg/kg

Group IV: Diabetic control (Diabetes was induced and no ginger or cinnamon extract given)

Group V: Diabetic treated with aqueous Ginger extract (200mg/kg)

Group VI: Diabetic treated with aqueous cinnamon extract (150mg/kg)

Group VII: Diabetic treated with Ginger (200mg/kg) and cinnamon (150mg/kg)

Group VIII: Diabetic treated with metformin (500mg/kg)

The animals were treated for 2 weeks with the ginger and cinnamon extracts while monitoring the fasting blood glucose level at 3-day intervals using Accucheck glucometer. The treatment was withdrawn for a period of 1 week during which the fasting blood glucose was still monitored at 3 day interval.

3.4 Induction of Diabetes mellitus

Diabetes mellitus was induced in the rats by a single intra-peritoneal injection of alloxan at a dose of 150mg/kg body weight after 12 hour of fasting. After 48 hours fasting blood glucose levels of the rats was measured using glucometer, and rats with fasting blood glucose levels of 11.1mmol/L or above are considered diabetic (Stanley and Venogopal, 2001).

3.5 Blood sample collection and processing

At the end of the 3 weeks experimental period the animals were sacrificed by humane decapitation. Blood sample was collected in plain containers. Serum was obtained from the

blood samples collected in plain containers by centrifugation at 3000g for 15 minutes in a bench centrifuge (Beckman Coulter). The serum was separated and kept in labeled bottles and kept at 0°C in a freezer for 2 days before the biochemical analysis were carried out.

3.6 Biochemical analysis

The sera collected for the various groups were used to estimate total cholesterol (Allain *et al.,* 1974), Triglycerides (Jacob and Demark,1966), HDL-c (Lopes-virella *et al.*,1977), LDL-c (Friedwald,1972), Urea (Patton and Crouch,1977), Creatinine (Bartels and Bohmer, 1972), sodium (Henry,1974), chloride (Fried, 1972) and potassium (Henry,1974).

3.6.1 HDL cholesterol determination by enzymatic CHOD-PAP method

Principle

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation the cholesterol concentration in the HDL fraction, which remains in the supernatant is determined as show below.

Procedure

Six centrifuge tubes were used for this assay, two were labeled sample/standard, another two were labeled precipitant R1 (Phosphotungstic Acid) and the last two labeled diluted precipitant R1(Phosphotungstic Acid diluted in distilled water in the ratio 4:1 respectively). The centrifuge tubes were divided into 2 groups macro and semi-micro .Five hundred microliter of sample/standard was added into the macro centrifuge while two hundred microliter was added to the sample/standard semi micro centrifuge tube. One thousand microliter R1 was added to the tube labeled precipitant while five hundred microliter of R2 was added to tube labeled diluted

precipitant. The mixture was the allowed to sit for 10 minutes at room temperature. It was then centrifuged for 10 minutes at 4000 rpm. The supernatant was separated and used to determine cholesterol using the CHOD-PAP method.

Three centrifuge tubes were used to in the assay for the HDL cholesterol from the supernatant. In a centrifuge tube one hundred microliters of distilled water was added, this served as the blank, one hundred microliters of supernatant of the sample was added, while in another tube one hundred microliters of supernatant of the standard was added, then one thousand microliters of the Cholesterol CHOD-PAP reagent was added to all three tubes. It was then incubated 5 minutes at 37°C. The absorbance of the sample(A_{sample}) and standard ($A_{standard}$) was then measured against the reagent blank at a wavelength of 500nm

Calculation

The concentration of HDL-cholesterol was calculated using the formula

$$\frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{Concentration of standard(mg/dl)}$$

3.6.2 Determination of serum triglyceride concentration by total enzymatic method

Principle

Triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipase}} \text{Glycerol} + \text{Fatty acids}$$

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

$$\text{Glycerol} - 3 - \text{phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone} + \text{phosphate} + H_2O_2$$

$$2H_2O_2 + 4-\text{aminophenazone} + 4-\text{chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine} + HCL + 4H_2O$$

Procedure

Three centrifuge tubes were used; they were labeled blank, standard and sample. For the blank one thousand microliters of the reagent R1 was added into the tube, for the standard ten microliters of the sample and one thousand microliters of the reagent R1 were added while for the standard ten microliters of the standard and one thousand microliters of the reagent R1 were added. R1 is an enzymatic reagent.

The mixture was incubated for 5 minutes at 37 °C and the absorbance of the sample and standard was measured at 500nm against the reagent blank in the colorimeter.

Calculation

The concentration of the triglycerides was calculated using the formula below

$$\frac{\Delta \text{ A Sample}}{\Delta \text{A Standard}} \times \text{Concentration of standard(mmol/l)}$$

3.6.3 Determination of serum total cholesterol by enzymatic hydrolysis

Principle

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestene} - 3 - \text{one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + 4-\text{aminoantipyrine} \xrightarrow{\text{HPO}} \text{quinoneimine} + 4H_2O_2$$

Procedure

Three centrifuge tubes were labeled reagent blank, standard and sample. To the tube labeled blank ten microliters of distilled water and one thousand microliters of the reagent was added. For the tube labeled standard; ten microliters of standard and one thousand microliters of the reagent was added while to the sample tube ten microliters of the sample was added as well as one thousand microliters of the reagent.

The mixture was incubated for 5 minutes at 37 °C. The absorbance of the sample and the standard were measured against the reagent blank at an absorbance of 500nm.

Calculation

$$\frac{\Delta \text{ A Sample}}{\Delta \text{A Standard}} \times \text{Concentration of standard(mg/dl)}$$

3.6.4 Determination of LDL concentration

LDL cholesterol level was calculated using this formula

LDL cholesterol = Total cholesterol –triglyderides(1/2.2) – HDL cholesterol(mg/dl)

3.6.5 Determination of serum sodium concentration using Trinder, 1951 and Maruna, 1958 principles

Principle

This is based on a modification of the methods described by Maruna (1958) and Trinder (1951) in which sodium is precipitated as triple salt, sodium magnesium uranyl acetate, the excess uranium I then reacted with ferrocyanide producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test sample.

Procedure

The test tubes were labeled blank, standard and sample.1.0 ml of filtrate reagent was added to all the test tubes.50µl of sample was then added to the sample test tube while 50µl of standard was added to the standard tube while 50µl of distilled water was added to the blank. The tubes were shaken vigorously for 3 minutes. They were then centrifuged at 1500rpm for 10 minutes. To 50µl of the supernatants of each of the tubes 1.0 ml of acid reagent was added. 50µl of color reagent was also added to all the tubes. The sample, blank and the sample were then read at 550nm.

Calculation

$$(Absorbance\ of\ blank - absorbance\ of\ sample) \times concentration\ of\ standard(mEq/l)$$

3.6.6 Determination of serum potassium level using Terry and Sesin, 1958 principles

Principle

The amount of potassium is determined by using sodium tetraphenylboron to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration in the range of 2-7mEq/L.

Procedure

The test tubes were labeled sample, standard and blank. 1ml of potassium reagent was added to all the tubes. 0.01ml of sample was added to the test tube sample while 0.01ml of standard was added to the test tube labeled standard. The tubes were allowed to stand for 3minutes at room temperature. The absorbance of the blank, sample and standard were read at 500nm.

Calculation

The concentration of the potassium was calculated using the formula

$$\frac{\Delta\ \text{A Sample}}{\Delta\text{A Standard}} \times \text{Concentration of standard(mEq/l)}$$

3.6.7 Determination of serum chloride level using Fried 1972 method

Principle

Chloride ions in serum react with a Hg-2,4,6-tri-(2-pyridyl)-1,3,5 triazine (TPTZ) complex. This reaction liberates TPTZ which then reacts with ferrous ion to form a blue Fe-TPTZ complex. The absorbance of this blue complex is proportional to the chloride concentration.

$Hg(TPTZ)_2^{2+} + 2Cl^- \text{----------} > Hg(Cl)_2 + TPTZ$

$2TPTZ + Fe^{2+} \text{----------} > Fe(TPTZ)_2^{2+}$

Procedure

Two centrifuge tubes were labeled sample and standard. To the tube labeled standard 5µl of standard and 1000µl of the reagent were added, while 5µl of the sample and 1000µl of the reagent was added.

The mixture was shaken and incubated at 37 °C for 5 minutes.

The absorbance was then read at 610nm. The concentration of chloride was then extrapolated from a curve plotted of absorbance of standard against that of the chloride.

3.6.8 Determination of serum Urea level by Patton and Crouch, 1977 principle

Principle

This is based on the reaction of urea with water to give ammonia and carbon dioxide.

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

The reagent contains salicylate and hypochlorite which reacts with the ammonia to form a green complex (2,2-dicarboxylindophenol)

Procedure

Three centrifuge tubes labeled blank, standard and sample were used. To the blank tube 1000µl of reagent 1(urease) was added, to the standard tube 10µl of the standard and 1000µl of the reagent 1 were added and to the sample tube 10µl of the standard and 1000µl of the reagent 1 were added. It was mixed and incubated for 3 minutes at 37 °C. Then 200µl of reagent 2 was added to all 3 tubes and incubated for 5 minutes at 37 °C and the absorbance was measured at 600nm in a spectrophotometer.

Calculation

The concentration of urea was calculated using the formula

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 8.33(\text{mmol/l})$$

3.6.9 Determination of serum Creatinine level by Bartels and Bohmer, 1972 principle

Principle

Creatinine reacts with picric acid in an alkaline solution to from a colored complex. The amount of colored complex formed is directly proportional to the creatinine concentration.

Procedure

In a test tube labeled standard macro 2ml of the creatinine reagent and 0.2ml of the standard were added while in a test tube labeled standard micro1ml of the reagent and 0.1ml of the standard were added. In a different test tube labeled sample macro 2ml of the reagent and 0.2ml of the sample were added while in the tube labeled sample micro 1ml of the reagent and 0.1ml of the sample were added. The mixtures were then shaken and the immediately the absorbance were read at 492nm in the spectrophotometer.

The concentration of the creatinine was calculated using the formula below

$$\frac{\Delta \text{ absorbance of sample}}{\Delta \text{ absorbance of standard}} \times 177\mu mol/l$$

$$\Delta\, absorbance = absorbance\ of\ macro - absorbance\ of\ micro$$

3.7 Determination of blood glucose by Glucose oxidase method

Principle

Glucose oxidase (GOD) converts Glucose into gluconate. The Hydrogenperoxide (H_2O_2) produced in the reaction is degraded by peroxidase (POD) and gives a colored product Phenol and 4-Aminoantipyrine which is measurable at 505 nm. The increase in absorbance correlates with the glucose concentration of the sample.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + phenol\ + 4 - aminoantipyrine \xrightarrow{POD} red\ qiunone\ + 4H_20$$

Procedure

To the blank test tube 1ml working reagent and 10µl distilled water were added, to the standard test tube 1ml working reagent and 10µl standard were added and lastly to the sample test tube 1ml working reagent and 10µl sample were added. They were then shaken and left to stand for 10 minutes at room temperature. The absorbance was then read at 505nm in the spectrophotometer.

Calculations

$$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times Concentration\ of\ standard = Concentration\ of\ sample$$

3.8 Statistical analysis

Serum glucose, lipids and electrolyte levels were compared between groups using one way ANOVA followed by Tukey's test in the instat statistical analysis software. Statistical significant difference was considered at $p<0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.2 Antidiabetic studies

Figure 4 summarized the results for the effect of oral administration of aqueous extracts of ginger rhizome, cinnamon bark and a combination of ginger and cinnamon extracts on fasting blood glucose of alloxan induced diabetic rats compared to normal control, diabetic control, and the group treated with metformin. There was significant ($p<0.01$) elevation in fasting blood glucose of diabetic control compared to the normal control and the non-diabetic group treated with ginger and cinnamon extracts. Diabetic groups treated with ginger extract, cinnamon extract and their mixture showed a significant ($p<0.01$) decrease in their fasting blood glucose compared to the diabetic control group. Diabetic groups treated with ginger extract, cinnamon extract and their mixture showed no significant ($p>0.05$) difference compared to the diabetic group treated with metformin. There is no significant difference ($p>0.05$) between groups treated with ginger extract and cinnamon extract. Comparison between non-diabetic groups treated with ginger extract and cinnamon extract showed no significant ($p >0.05$) difference. Diabetic group treated with ginger extract showed significant ($p<0.05$) difference compared to non-diabetic group treated with ginger extract, also diabetic group treated with cinnamon extract compared to non-diabetic group treated with cinnamon showed a significant ($p< 0.05$) difference. Diabetic group treated with metformin when compared to the diabetic group treated with a combination of ginger and cinnamon showed no significant ($p>0.05$) difference.

4.1.3 Effect of withdrawal of treatment

Figure 5 summarized the results for the effect of withdrawal of aqueous extract ginger; cinnamon and their combination on fasting blood glucose of alloxan induced diabetic rats. There is significant ($p<0.05$) difference between diabetic control group compared to diabetic groups treated with metformin, ginger extract, cinnamon extract and their combination. However there is no significant difference between the diabetic group treated with metformin and that treated with a combination of ginger and cinnamon extracts.

4.1.4 Percentage glucose loss

Figure 6 shows the percentage glucose loss for the various groups. This shows the percentage of blood glucose loss compared to the control.

4.1.5 Effect of treatment of diabetic rats with ginger, cinnamon and their combination on lipds

Figure 7 shows the effect of treatment of alloxan induced diabetic rats with ginger, cinnamon and their combination. There is a significant increase ($p<0.05$) in serum lipids of diabetic control group compared to the control. Comparison of total cholesterol and triglycerides in diabetic control group and diabetic groups treated with treated with cinnamon, ginger and their combination showed a significant decrease.

4.1.5 Effect of treatment of diabetic rats with ginger, cinnamon and their combination on electrolytes

Figure 8 shows the effect of treatment of diabetic rats with ginger, cinnamon and their combination on electrolytes. There is no significant ($p>0.05$) difference in sodium, potassium and chloride levels in the control compared to all the groups.

4.1.5 Effect of treatment of diabetic rats with ginger, cinnamon and their combination on urea and creatinine

Figure 9 shows the urea and creatinine levels in diabetic rats administered aqueous extract of Ginger rhizome, Cinnamon bark, and a combination of both ginger and cinnamon of diabetic rats compared to the control. No significant difference ($p>0.05$) is seen between the normal , normal treated with Ginger extract, normal treated with Cinnamon extract, compared to diabetic groups treated with cinnamon, ginger and a combination of cinnamon and ginger.

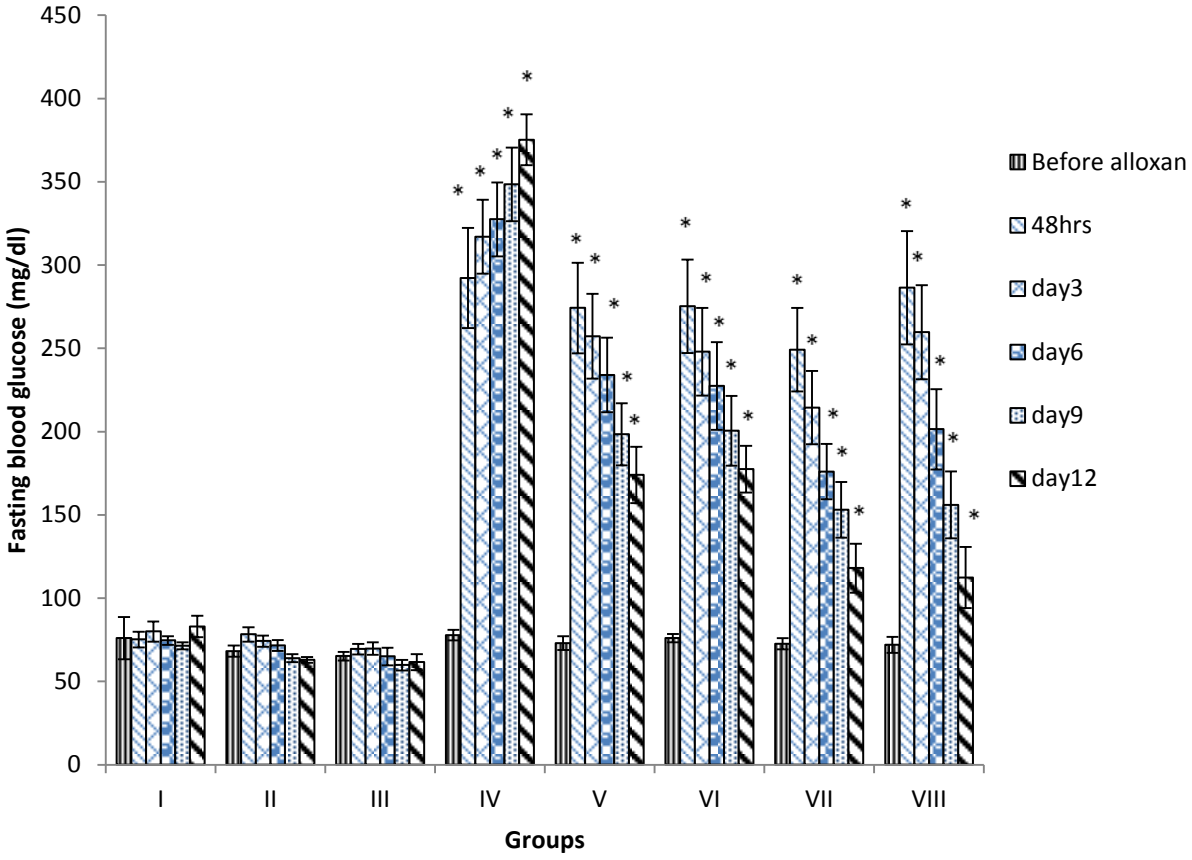

Fig 4: Fasting Blood glucose in diabetic rats orally administered with aqueous extract of Ginger rhizome, Cinnamon bark and a combination of both Ginger and Cinnamon extracts.
Bars with * are significantly different from the control at $p < 0.05$
Values are expressed as Mean ±SEM

Key
Group I: Normal
Group II: Non-diabetic group given ginger extract at a dose of 200mg/kg
Group III: Non-diabetic group given cinnamon extract at a dose of 150mg/kg
Group IV: Diabetic control (Diabetes was induced and no ginger or cinnamon extract given)
Group V: Diabetic treated with aqueous Ginger extract (200mg/kg)
Group VI: Diabetic treated with aqueous cinnamon extract (150mg/kg)
Group VII: Diabetic treated with Ginger (200mg/kg) and cinnamon (150mg/kg)
Group VIII: Diabetic treated with metformin (500mg/kg)

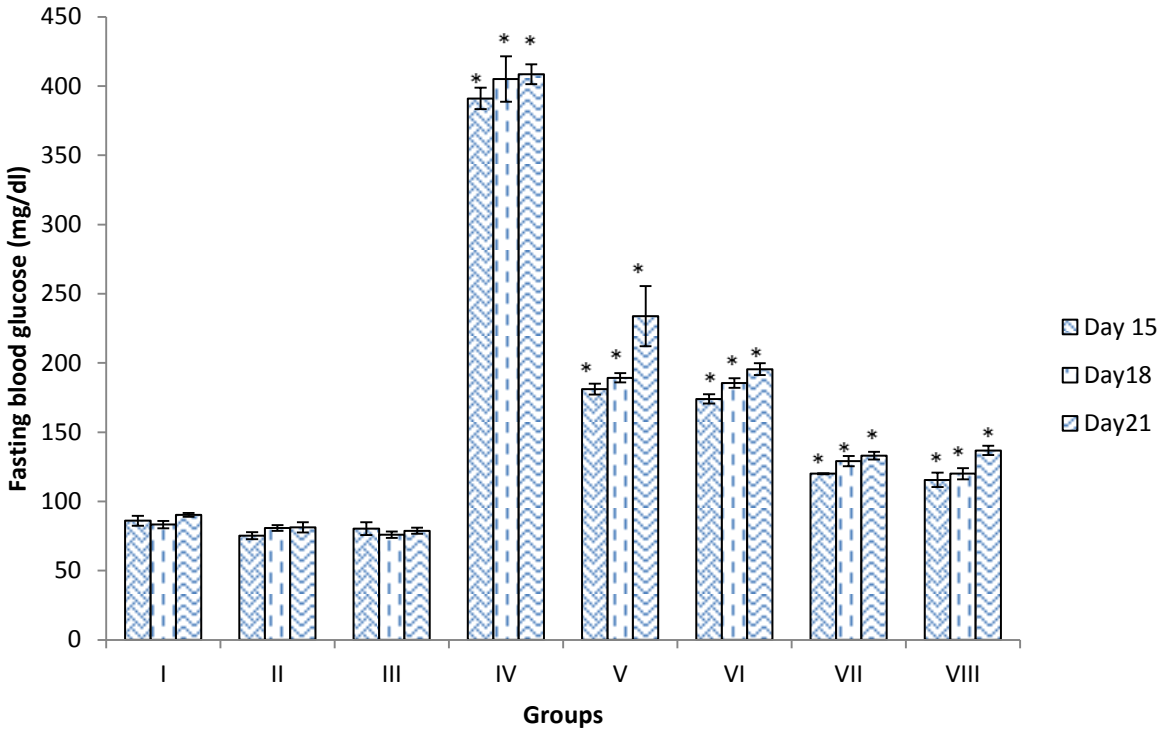

Fig 5: Fasting Blood glucose in diabetic rats withdrawn from oral administration with aqueous extract of Ginger rhizome, Cinnamon bark and a combination of both Ginger and Cinnamon extracts

Bars with * are significantly different from the control at $p<0.05$
Values are expressed as Mean ±SEM

Key
Group I: Normal
Group II: Non-diabetic group given ginger extract at a dose of 200mg/kg
Group III: Non-diabetic group given cinnamon extract at a dose of 150mg/kg
Group IV: Diabetic control (Diabetes was induced and no ginger or cinnamon extract given)
Group V: Diabetic treated with aqueous Ginger extract (200mg/kg)
Group VI: Diabetic treated with aqueous cinnamon extract (150mg/kg)
Group VII: Diabetic treated with Ginger (200mg/kg) and cinnamon (150mg/kg)
Group VIII: Diabetic treated with metformin (500mg/kg)

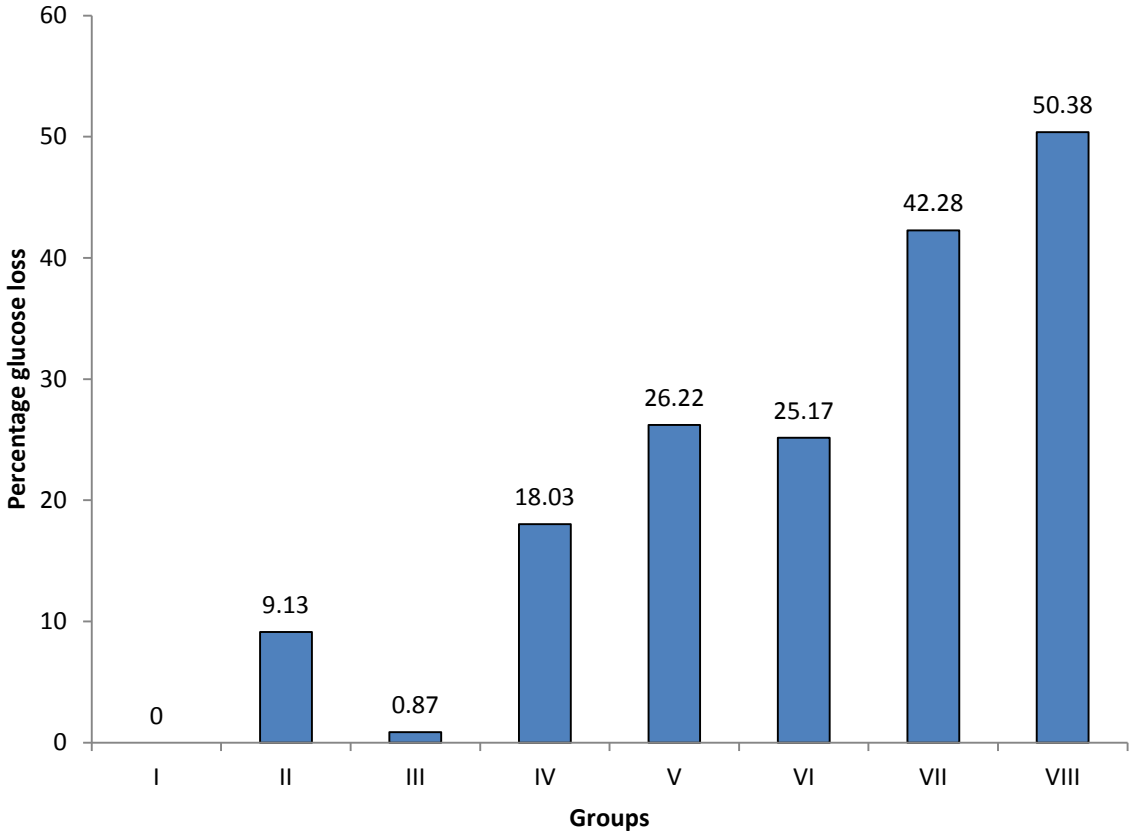

Fig 6: Percentage glucose loss on all the groups.

Key
Group I: Normal
Group II: Non-diabetic group given ginger extract at a dose of 200mg/kg
Group III: Non-diabetic group given cinnamon extract at a dose of 150mg/kg
Group IV: Diabetic control (Diabetes was induced and no ginger or cinnamon extract given)
Group V: Diabetic treated with aqueous Ginger extract (200mg/kg)
Group VI: Diabetic treated with aqueous cinnamon extract (150mg/kg)
Group VII: Diabetic treated with Ginger (200mg/kg) and cinnamon (150mg/kg)
Group VIII: Diabetic treated with metformin (500mg/kg)

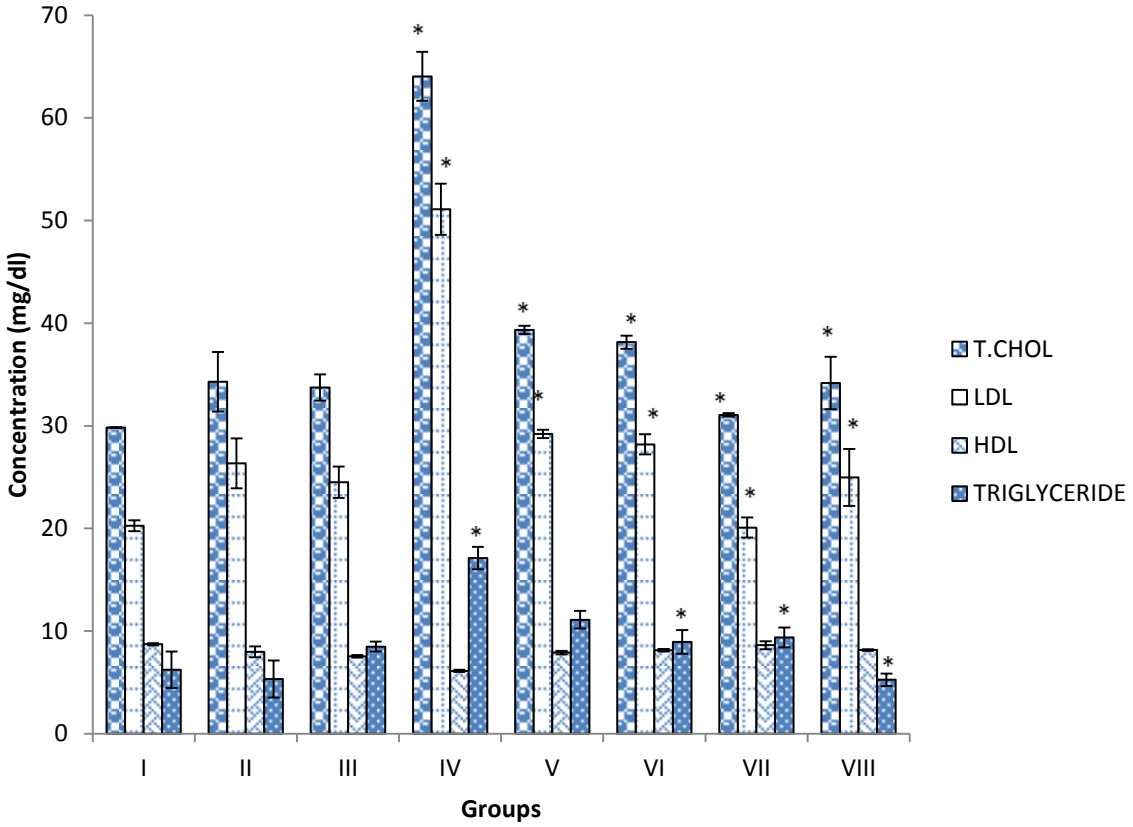

Fig 7: Lipid profile of Alloxan induced diabetic rats orally administered with aqueous extract of Ginger rhizome, Cinnamon bark and a combination of both Ginger and Cinnamon

Values are expressed as Mean ±SEM

Key
Group I: Normal
Group II: Non-diabetic group given ginger extract at a dose of 200mg/kg
Group III: Non-diabetic group given cinnamon extract at a dose of 150mg/kg
Group IV: Diabetic control (Diabetes was induced and no ginger or cinnamon extract given)
Group V: Diabetic treated with aqueous Ginger extract (200mg/kg)
Group VI: Diabetic treated with aqueous cinnamon extract (150mg/kg)
Group VII: Diabetic treated with Ginger (200mg/kg) and cinnamon (150mg/kg)
Group VIII: Diabetic treated with metformin (500mg/kg)

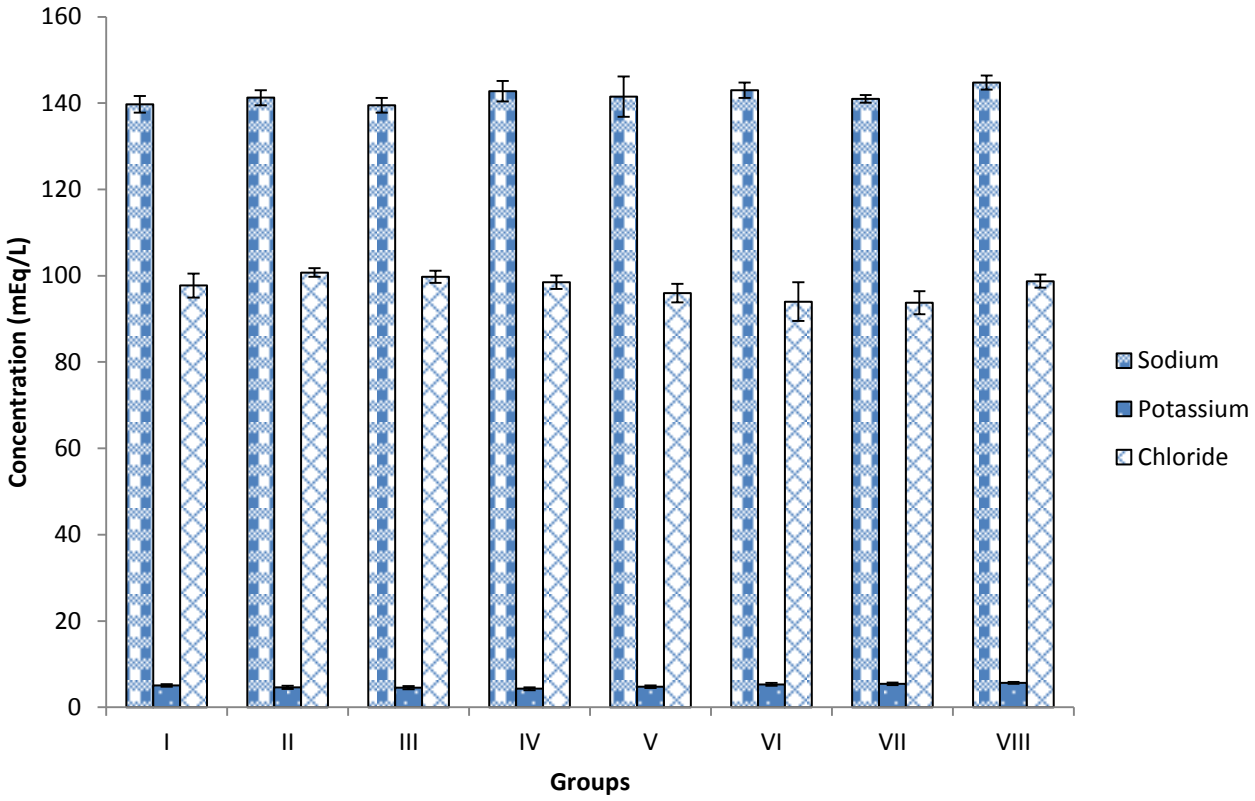

Fig 8: Electrolytes in diabetic rats orally administered with aqueous extract of Ginger rhizome, Cinnamon bark and a combination of both Ginger and Cinnamon extracts.

Values are expressed as Mean ±SEM

Key
Group I: Normal
Group II: Non-diabetic group given ginger extract at a dose of 200mg/kg
Group III: Non-diabetic group given cinnamon extract at a dose of 150mg/kg
Group IV: Diabetic control (Diabetes was induced and no ginger or cinnamon extract given)
Group V: Diabetic treated with aqueous Ginger extract (200mg/kg)
Group VI: Diabetic treated with aqueous cinnamon extract (150mg/kg)
Group VII: Diabetic treated with Ginger (200mg/kg) and cinnamon (150mg/kg)
Group VIII: Diabetic treated with metformin (500mg/kg)

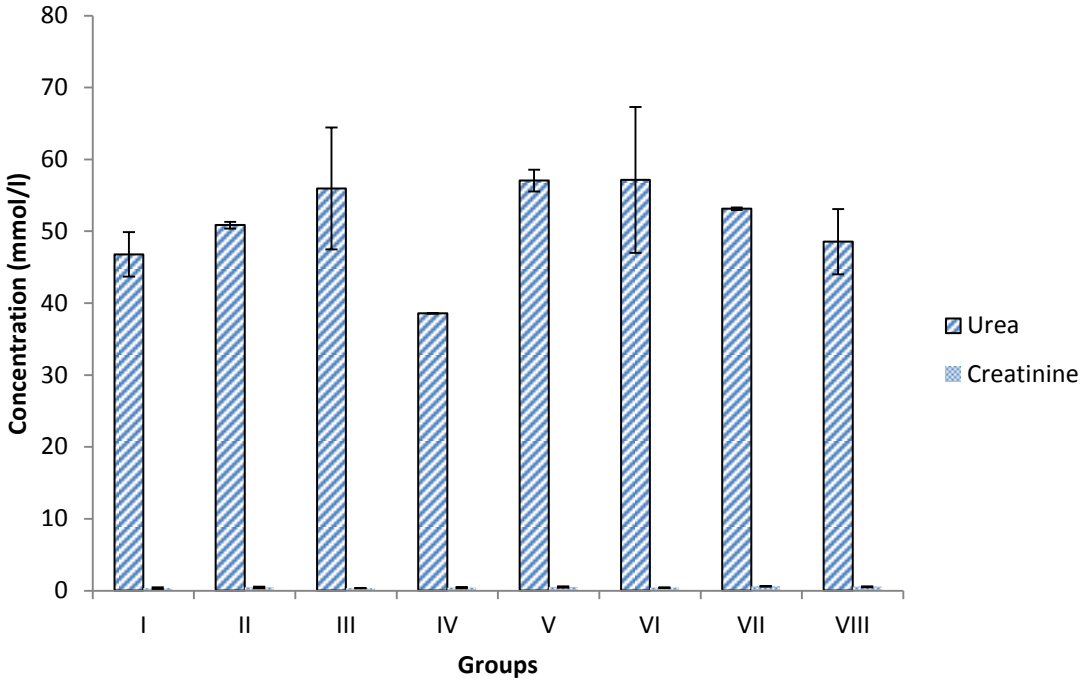

Fig 9: Urea and Creatinine in diabetic rats orally administered with aqueous extract of Ginger rhizome, Cinnamon bark and a combination of both Ginger and Cinnamon extracts.

Values are expressed as Mean ±SEM

Key
Group I: Normal
Group II: Non-diabetic group given ginger extract at a dose of 200mg/kg
Group III: Non-diabetic group given cinnamon extract at a dose of 150mg/kg
Group IV: Diabetic control (Diabetes was induced and no ginger or cinnamon extract given)
Group V: Diabetic treated with aqueous Ginger extract (200mg/kg)
Group VI: Diabetic treated with aqueous cinnamon extract (150mg/kg)
Group VII: Diabetic treated with Ginger (200mg/kg) and cinnamon (150mg/kg)
Group VIII: Diabetic treated with metformin (500mg/kg)

4.2 Discussion

4.2.1 Anti-diabetic effects

The blood glucose lowering effect of aqueous extract of ginger rhizome observed may be due to the presence of phenols in the ginger. These phenols are found in the pungent components of ginger rhizome such as gingerols (Ovesen, 2012). Shagoals are phenolic compounds found in ginger which is dried or semi-dried, they are produced from gingerols upon drying, and this is because gingerols are heat labile. Khulood, (2014) showed that a compound in ginger 6-gingerol showed antidiabetic properties. The 6-gingerol is also found to reduce fasting blood sugar significantly and improve glucose tolerance when administered at a dose of 100mg/kg for 12 days (Singh *et al.*, 2009). Two important enzymes associated with carbohydrates metabolism in diabetes are α-amylase and α-glucosidase, ginger extract show very high α-glucosidase and α-amylase inhibitory activities, the action of ginger rhizome extract against these two enzymes found to be correlated with the phenolic contents of gingerol and shogaol in these extracts (Rani *et al*., 2011). In *vivo* studies on rats showed that after long-term (8 weeks) feeding with ginger rhizome, the activities of pancreatic lipase, amylase, trypsin, and chymotrypsin were significantly increased (Platel and Srinivasan, 2000).

Diabetic group treated with cinnamon bark extract showed a significant reduction in fasting blood sugar compared to the diabetic control. This result is in agreement with studies carried out by Lee *et al.*, (2013) which showed that administration of cinnamon at 20mg/kg was found to help glycemic control and improve insulin secretion in diabetic patients. Studies also showed that it is possible that the reduction of oxidative stress and pro-inflammatory activities in the pancrease may protect the β-cells. Aqueous extract of cinnamon was shown to decrease alanine absorption hence reducing the blood sugar level because alanine plays a vital role in

gluconeogenesis; decrease in alanine suggests alterations in substrate availability such as utilization of available glucose in the blood (Kreydiyyeh *et al.,* 2000). This is one of the possible mechanisms through which cinnamon decreases blood sugar. Cinnamon is also showed to contain flavonoids and saponins as some of its phytochemical constituents. Flavonoids in the extract may have stimulating effect on insulin secretion from the remaining β-cells not completely destroyed by alloxan. However the gradual increase in glucose concentration post withdrawal of treatment with cinnamon suggests that the effect of the extract may not be curative.

The diabetic group treated with a combination of ginger (200mg/kg) and cinnamon (150mg/kg) showed a significant decrease in blood glucose. This may be attributed to the presence of flavonoids in both cinnamon and ginger that inhibit glucose-6-phosphatase activity in the liver thereby suppressing gluconeogenesis and glycogenolysis and consequently reduce hyperglycemia (Chen *et al.,* 1998). Therefore the anti-diabetic activity of the two plants studied maybe a combination of decreased glucose-6-phosphatase activity and possible regeneration of pancreatic cells (Youn *et al.,* 2004). The hypoglycemic effect of the combination of these two plants is as significant as that of metformin treated group. Different mechanisms of action of anti-diabetic plants have been proposed such as inhibition of intestinal glucose absorption (Youn *et al.,* 2004), correction of insulin resistance (Hu *et al.,* 2003), inhibition of hepatic glucose production (Eddouks *et al.,* 2003) and potentiation of insulin effect either by increasing the pancreatic secretion of insulin from the β cells (Pari and Amarnath, 2004).

4.2.2 Hypolipidemic

Diabetic dyslipidemia as a metabolic abnormality occurs in diabetes mellitus frequently and is associated with high triglycerides, low HDL cholesterol and high LDL cholesterol level

(Mooradian, 2009). It is showed that the most common recognized abnormality was high triglyceride, very low HDL cholesterol and high LDL cholesterol in Streptozotocin induced diabetic rats (He *et al.*, 2015). This is consistent with this study, where triglyceride and LDL cholesterol significantly increased, and HDL-cholesterol significantly decreased, after alloxan induction of diabetic rats. From the results in Fig 6 it can be seen that the serum level of total cholesterol in diabetic control group is highest and significantly different from all the other groups, same goes for triglyceride which is higher in diabetic control group than the rest of the groups. The LDL-cholesterol level is significantly greater in Diabetic control group while the level of HDL is higher in the normal group. Bhandari *et al.,* (2005) showed that ethanolic extract of ginger produced significant decrease in serum total cholesterol, LDL- cholesterol and triglycerides levels and increased HDL-cholesterol level to diabetic rats, and the extract protected the tissues from lipid peroxidation.

Flavonoids are some of the phytochemical constituents found in both ginger rhizome and cinnamon bark. These flavonoids are reported to protect the cells through attenuation of lipid peroxidation and decreased production of free radicals thereby offering protection against oxidative stress by scavenging of free radicals. (Nwanjo, 2007). Hayashi *et al.,* (2002) showed that the presence of polyphenols in plant extracts have numerous protective health benefits which include lowering of blood lipids, also methoxylated flavonoids have the ability to suppress Apo B containing lipoprotein secretion, this in turn helps in modulating liver lipid metabolism. Apo lipoprotein B is the primary transport protein for LDL, and high levels of this apo protein are found to be related to heart disease.

The hypolipidemic activities of ginger as reported by Srinivasan and Sambaiah (1991) is by stimulating the conversion of cholesterol to bile acids, an important pathway of elimination of

cholesterol from the body. Han *et al*., (2005) found that *Z. officinale* increased the faecal excretion of cholesterol, suggesting that ginger may block absorption of cholesterol in the gut. Moreover, Afshari *et al*., (2007), Nammi *et al*., (2009) and Ramudu *et al*., (2011**)** mentioned that the hypocholesterolemic effect of ginger may be attributed to inhibition of cellular cholesterol synthesis which results in augmenting the LDL receptor activity, leading to the elimination of LDL from plasma thus modifying lipoprotein metabolism.

4.2.3 Effect on Electrolytes, Urea and Creatinine

Electrolytes play an important role in many body processes, such as controlling fluid levels, acid-base balance (pH), nerve conduction, blood clotting and muscle contraction. Potassium, sodium and calcium are all important for proper electrolyte balance. Electrolyte imbalance resulting from kidney failure, dehydration, fever, and vomiting has been suggested as one of the contributing factors toward complications observed in diabetes and other endocrine disorders. (Husain *et al.,* 2009). From this study it can be seen that there is no significant difference seen in the levels of the sodium, potassium and chloride between group 1 and the remaining seven groups, similarly the difference between the groups is not significant. The values for sodium, potassium and chloride fall within the normal range according to John-Delaney (1996). This shows that neither diabetes mellitus nor the administration of the ginger and cinnamon extracts caused any significant alteration on the reabsorption capacity of the kidneys. The established markers of Glomerular Filteration Rate (GFR) are plasma urea and creatinine, however plasma creatinine is a more sensitive index of kidney function compared to plasma urea. This is because creatinine fulfills most of the requirement for a perfect filteration marker. (Perrone *et al.,* 1992).

Mitch and Walser, (1986) reported that a graph of creatinine plotted against time will give a straight line, this type of graph can be used to determine how fast or otherwise a patient is losing

kidney function. An extrapolation can be made from the graph to give a rough idea when the kidney will fail completely and initiate dialysis. It can also be used to measure the progress of treatment is case of renal disease. Plasma creatinine is also helpful in recognizing when there is an acute drop in kidney function as well as used in monitoring of disease progression.(Mitch and Walser, 1986) It was observed from this study that the levels of urea and creatinine were found to be unaffected by neither diabetes nor the treatment. This however is not in accordance with the study carried out by Idonije *et al.,* (2011). This may be due to the difference in the specie or duration of the study, because the study was done over a little period of time while that of the study carried out by Idonije *et al.*, (2011) is on human subjects who might have had the disease for a very long period of time.

CHAPTER FIVE

5.1 Summary

The potency of the aqueous extract of Ginger rhizome, Cinnamon bark and a combination of ginger rhizome and cinnamon bark on diabetic induced rats was compared in order to determine which is most potent. In the analysis done it was found that a combination of Ginger and cinnamon showed more potency as it was found to lower the glucose level to a level as low as that of the group treated with metformin, however both Cinnamon and Ginger showed significant reduction in blood glucose level of the alloxan induced diabetic rats, with cinnamon showing higher anti-diabetic activity than Ginger.

5.2 Conclusion

Results from this study suggest that aqueous extract of Ginger rhizome and Cinnamon bark has hypoglycemic and hypolipidemic properties in rats. The highest hypoglycemic activity was seen in the group administered a combination of ginger and cinnamon suggesting that this combination may be very effective in management of diabetes mellitus.

5.3 Recommendation

Based on the findings of this research, the following recommendations are made:

1. Consumption of both ginger and cinnamon should be encouraged especially in local community.

2. Further studies on different extracts of both cinnamon bark and ginger rhizome to find out their effects on diabetes mellitus and its complications are recommended.

3. Variations on the composition of Ginger and cinnamon from various locations could be studied.

4. Further investigation to be carried out on the compounds found in both ginger and cinnamon to ascertain which confer its therapeutic properties.

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APPENDIX I

Effect of oral administration of cinnamon, ginger and a combination both on fasting blood glucose of Alloxan induced diabetic rats.

Group	Before Alloxan	48 hours	Day 3	Day 6	Day 9	Day 12
1	76.00±12.75	75.20±4.67^{abcde}	80.00±6.03^{fghij}	74.60±2.65^{klmno}	71.4±2.14^{pqrst}	83.00±6.46^{uvwx}
2	68.20±3.40	78.30±4.31^{abcde}	74.20±3.39^{fghij}	71.60±3.25^{klmno}	64.00±2.37^{pqrst}	63.00±1.73^{uvwx}
3	65.20±2.63	69.40±3.23^{abcde}	69.69±3.75^{fghij}	65.00±5.25^{klmno}	59.80±3.15^{pqrst}	61.60±4.78^{uvwx}
4	77.80±3.13	292.20±30.01^{a}	317.00±22.20^{f}	327.00±22.26^{k}	348.40±22.04^{pqrst}	375.20±15.31^{uvwx}
5	73.00±4.10	274.40±27.25^{ab}	257.2±25.47^{g}	234.00±22.43^{l}	198.40±18.57^{pq}	174.00±16.93^{uv}
6	76.00±2.45	275.20±28.02^{ac}	248.00±26.31^{h}	227.40±26.27^{m}	200.49±20.87^{pr}	177.40±13.98^{uw}
7	72.60±3.33	249.20±28.02^{ad}	214.40±21.96^{i}	176.00±16.62^{n}	153.00±16.74^{pr}	118.00±14.41^{ux}
8	72.00±4.88	286.40±34.00^{ade}	259.80±28.29^{j}	201.40±24.11^{0}	156.80±20.03^{pt}	112.40±18.34^{uvwx}

Values are expressed as mean ±SEM, n=4

APPENDIX II

Effect of withdrawal of treatment on alloxan induced diabetic rats

GROUP	DAY 15	DAY 18	DAY 21
1	86.00±3.63	83.25±2.66	90.25±1.38
2	75.25±2.63	80.75±2.10	81.00±3.70
3	80.25±4.66	76.00±2.35	78.75±2.18
4	391.00±7.71*	405.00±16.47*	408.50±7.08*
5	181.00±3.94*	189.25±3.38*	233.75±21.65*
6	174.00±3.34*	184.50±3.43*	195.50±4.35
7	120.00±0.41*	129.50±3.70*	133.00±2.86*
8	115.50±5.17*	120.00±4.10*	136.75±3.35*

Values are expressed as mean ±SEM, n=4

APPENDIX III

Effect of aqueous extract of cinnamon, ginger and a combination of both on lipid profile of alloxan induced diabetic rats.

	T.CHOL	LDL	HDL	TRIGLYCERIDE
GP1	29.82±0.04	20.26±0.52	8.72±0.11	6.23±1.77
GP2	34.30±2.90*	26.35±2.43*	7.99±0.53	5.32±1.81
GP3	33.73±1.29*	24.50±1.52*	7.53±0.13	8.48±0.48
GP4	60.04±2.39	51.09±2.49	6.12±0.12*	17.19±1.09*
GP5	39.33±0.40*	29.21±0.41*	7.90±0.18	11.1±0.86*
GP6	38.14±0.63*	28.19±0.98*	8.14±0.12	8.99±1.15
GP7	31.06±0.17*	20.08±0.98*	8.63±0.39	9.39±0.97
GP8	34.17±2.55*	24.96±2.77*	8.15±0.09	5.25±0.61

Values are expressed as mean ±SEM, n=4

APPENDIX IV

Effect of aqueous extract of Ginger, Cinnamon, and a combination of both on Electrolytes, Urea and Creatinine in alloxan induced diabetic rats.

	Sodium(mEq/L)	Potassium(mEq/L)	Chloride(mEq/L)	Urea	Creatinine
Group 1	139.75±1.93	5.07±0.27	97.75±2.78	46.79±3.09	0.35±0.13
Group 2	141.25±1.75	4.62±0.39	100.75±1.03	50.85±0.48	0.45±0.11
Group 3	139.50±1.71	4.55±0.38	99.75±1.38	55.96±8.46	0.35±0.03
Group 4	142.75±2.39	4.32±0.34	98.50±1.55	38.59±0.05	0.43±0.08
Group 5	141.50±4.69	4.80±0.29	96.00±2.16	57.05±1.52	0.52±0.09
Group 6	143.00±1.78	5.30±0.33	94.00±4.50	57.14±10.17	0.42±0.05
Group 7	141.25±0.89	5.42±0.29	93.75±2.65	53.15±0.18	0.60±0.04
Group 8	144.75±1.65	5.67±0.25	98.75±1.49	48.55±4.52	0.55±0.06

Values are expressed as mean ±SEM, n=4

Appendix V

Weight , food intake and water intake.

Groups	Initial body weight(g)	Final body weight(g)	Daily feed intake(g)	Daily water intake(ml)
1	62.25±4.03	274.00±27.76	110.52±2.53	409.20±12.14
2	62.75±5.25	227.50±32.07	105.44±5.20	429.41±17.58
3	62.50±7.14	214.75±23.51	104.76±12.09	397.67±12.90
4	63.75±9.74	176.25±12.06	133.56±3.98	498.09±23.00
5	60.25±7.14	210.00±14.39	110.84±15.20	462.12±8.97
6	65.75±12.00	249.50±38.17	106.20±10.40	425.80±12.01
7	64.50±16.68	227.75±30.74	111.32±8.09	408.63±3.09
8	62.75±11.29	220.05±7.90	102.64±7.40	417.56±7.09

Value are expressed as mean ±SD