STUDIES ON THE HYPOGLYCAEMIC PROPERTIES OF THE METHANOLIC LEAF EXTRACT OF *TACAZZEA APICULATA* (OLIV.) ON ALLOXAN-INDUCED DIABETIC RATS

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MARCH, 2016

DEDICATION

This dissertation is dedicated to Allah (swt) and my Ever-Loving Parents, AlhajiAttahiru A. Umar and Zainab O. Umar, for their unwavering Love, Support and Encouragements. May Allah lengthen our stay together and reward you with Janaat, Amin.

DECLARATION

Ideclare that the work in this dissertation entitled"STUDIES ON THE HYPOGLYCAEMICPROPERTIES OF THE METHANOLIC LEAF EXTRACT OF *TACAZZEA APICULATA* (OLIV.) ON ALLOXAN-INDUCED DIABETIC RATS"has been performed by me in the Department of Biological Sciences under the supervision of Prof. S.P. Bako and Prof. A.K. Adamu. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

UMAR, Ohunene Fatimah _____ _____

Signature Date

CERTIFICATION

This dissertation titled "STUDIES ON THE HYPOGLYCAEMIC PROPERTIES OF THE METHANOLIC LEAF EXTRACT OF *TACAZZEA APICULATA* ON ALLOXAN-INDUCED DIABETIC RATS" meets the regulations governing the award of degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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Prof. A.K. Adamu Member, Supervisory Committee Dept. of Biological Sciences ABU, Zaria	Signature	Date
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ABSTRACT

An investigation on the hypoglycaemic effects of the methanolic leaf extract of *Tacazzeaapiculata* on alloxan-induced diabetic rats was conducted. The dried leaves of *Tacazzeaapiculata* was extracted with 70% methanol. Alloxan was injected intraperitoneally as a single dose of 150mg/kg b.w. for diabetes induction in the rats. Glibenclamide was used as a standard anti diabetic drug. The effect of the orally administered extract (100, 200 and 400mg/kg body weight) and Glibenclamide (standard drug) (5mg/kg b.w.) on blood glucose level, body weight, kidney and lipid profile were examined in the diabetic rats. The extract and the standard drug Glibenclamide were orally administered daily for 28 days to alloxan-induced diabetic rats. The fasting blood glucose levels were determined at intervals of one week. At the end of the 28 days, blood samples were collected from all groups and assayed spectrophotometrically for serum concentrations of Total Cholesterol (TC), Triglyceride (TRIGS), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), urea and creatinine. Preliminary phytochemical screening revealed the presence of flavonoids, saponins, tannins, alkaloids, terpenoids and triterpenes in the leaves of *Tacazzeaapiculata*. The toxicity study of the extract showed its safety level up to 5000mg/kg b.w., implying that it is safe to apply to humans. The study showed a significant reduction ($p<0.01$) in the fasting blood sugar levels in alloxan-induced diabetic rats treated with 100, 200 and 400mg/kg b.w. of the extract for 4 weeks post treatment with a maximum reduction recorded in the groups treated with 100mg/kgb.w. There was a significant decrease ($p<0.05$) in the serum levels of TC, TRIGS, LDL-C and an increase in HDL-C in treated diabetic rats¸ with a higher reduction observed in the groups treated with extract 100mg/kg b.w. for TC, TRIGS,LDL-C. Marked improvement in HDL-C was observed with 100mg/kg b.w. dose of the extract. At the end of the 4 weeks treatment, extract and Glibenclamide significantly prevented the rise in blood urea and serum creatinine levels as compared to the controls. It was concluded that the plant extract was capable of ameliorating hyperglycaemia in alloxan-induced diabetic rats and could be a potential source of new orally active agent for anti-diabetic therapy.

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CHAPTER ONE

1.0 INTRODUCTION

Diabetes Mellitus (DM) is the most severe and challenging metabolic pandemic of the 21st century because it affects essential biochemical activities in almost every cell in the body and increases the risk of cardiac and renal disorders. It is estimated that approximately 285million people worldwide or 6.6% of adults have diabetes, 70% of whom live in low-income and middle-income countries. This number is expected to increase by more than 50% in the next 20 years, if preventive programmes are not put in place. By 2030, some 483million people, or 7.8% of the adult population, are projected to have diabetes. The largest increases will take place in regions dominated by developing economies (International Diabetes Federation, 2009). This pandemic is characterized by excessive blood glucose due to deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced to control blood glucose (Davis and Granner, 1996). This disorder affects carbohydrate, protein and fat metabolism (Davis and Granner, 1996) and chronic hyperglycemia causes glycation of body proteins that in turn leads to secondary complications that affects eyes, kidneys, nerves and arteries (Sharma, 2003). The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy, nephropathy, and neuropathy. People with diabetes are at an increased risk of cardiovascular diseases.The treatment of DM is based on oral hypoglycemic agents and insulin injections.

Diabetes is a chronic disorder in the metabolism of carbohydrate, protein and fat, causedby defects in insulin secretion, insulin action or both (Devlin, 1997; Barar, 2000). It also leads to decreased physical activity, increased obesity, stress and changes in food consumption (Edwin*et al*, 2007). Diabetes is characterized by polyuria, polydipsia, and weight loss despite polyphagia, hyper glycaemia, glycosuria, ketosis, acidosis and coma (Ganong, 2003). Elevated insulin levels are not only symptoms of diabetes, but also of heart disease, peripheral vascular disease, stroke, high blood pressure, cancer, and obesity.

Majority of cases of diabetes fall into two broad categories; type I diabetes, caused by absolute deficiency of insulin secretion and type II (more prevalent category), is caused by combination of resistance to insulin action and inadequate compensatory insulin-secretory response (American Diabetes Association, 2005). Regardless of the type of diabetes, patients are required to control their blood glucose with medications and/or by adhering to an exercise programme and a dietary plan (Jarald*et al*, 2008).Insulin therapy by injection is given to those with type I DM and also to some patients with type II DM when oral hypoglycaemic drugs fail to lower blood glucose (Alam*et al*, 2003).Due to lifestyle modernization, non-insulin dependent diabetes mellitus is becoming a major health problem in developing countries (Jarald*et al*, 2008).

Oral antidiabetic agents are prescribed for patients with type II diabetes if diet and exercise fail to control glucose at desired level. Oral hypoglycemic agents such sulfonylurea, thiazolidinediones, and biguanides have many drawbacks, ranging from development of resistance and adverse effects to lack of responsiveness in large segment of population. According to statistics, Sulfonylureas lose effectiveness in 44% of patients within six years. Also, the treatments are associated with some side or even toxic effects. Thiazolidinedione may cause liver toxicity; sulfonylureas might worsen heart disease, lower the glucose below the normal range and increase body weight gain (Dey *et al*., 2002; DeFronzo, 2002; Michael *et al.,* 2005). Moreover, none of these glucose-lowering agents adequately controls the hyperlipidemia that is frequently met with the disease (Derek, 2001).

Plants are an integral part of human civilization. Medicinal plants are various plants thought by some to have various medicinal properties, but few plants or their phytochemical constituents have been proven by rigorous science to have medicinal effects. Medicinal plants are also been relied upon by over 80% of the world population for their basic health care needs (WHO, 2002). Finding healing powers in plants is thus an ancient idea. People on

all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory (Stockwell, 1988). It is estimated that there are 250,000 to 500,000 species of plants on Earth (Borris, 1996). Relatively small percentages (1 to 10%) of these are used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes (Moerman, 1996). The use of plants and plant extracts for medicinal purposes has been going on for thousands of years; and it has been the source of much useful therapy in both herbalism and folk medicine (Foye, 1999). The use of medicinal plants in traditional medicine has also generated a lot of interest and concern about their efficacy and safety margin, since 65-70% of the Nigerian population patronizes traditional medicine practitioners in their various forms and methods (Bubayero, 1998; Sofowora, 2001). Plants produce many chemical compounds, which have potential value in the treatment of diseases. Chemical compounds with beneficial effects have been isolated and biologically assayed to establish their medicinal activity. Modern drugs used in orthodox medicine have also been sourced from plants (Sofowora, 2001). It is therefore not surprising that medicinal plants are vastly employed in the treatment of various ailments which include; snake-bite, eye injuries, conjunctivitis, burns, scalds, abdominal colic, peptic ulcer, diarrhea, dysentery, chronic ulcer, measles, hepatitis, arthritis and rheumatism (Esuoso and Odetokun, 2005).

Looking back upon the last 2000 years of the history of medicine mankind has mainly used plants as the best source of medicine. Over 248,000 species of higher plants have been identified and from these 12,000 plants are known to have medicinal properties. The significance of research on natural medicinal plants is gaining momentum owing to its immense potential for improving the health care sector around the globe. The World Health Organization estimated that about 80% of Earth's inhabitants rely on traditional medicine for their primary health care needs that primarily involves the use of plant extracts or their active components. A concerted research is currently in vogue for understanding the

mechanism of action of these traditional procedures. With the sharp rise in popularity of traditional medicine, the economic importance of these plants has increased enormously. The role of medicinal plants is particularly important on the African continent. The continent is richly endowed with a variety of plant species, many of which have medicinal properties.

Plants are well known in traditional herbal medicine for their hypoglycemic activities, and available literature indicate that there are more than 800 plants species showing hypoglycemic activity (Rajagopal*et al*, 2008). One of such plant is *Tacazzea apiculata,* a latex producing, semi woody or climbing liana, several meters high with paired follicle fruits and cream-white edible flowers. Aqueous extracts of the leaves is used in folk lore medicine for its high anti convulsion activity in children, the white latex is used in treatment of cutaneous infections, wounds and boils, the leaves and it's latex is used by local fishermen to attract fish, and the leaves concoction is used in the treatment of diabetes.

1.1 Statement of Research Problem

*Tacazzea apiculata,*craw-craw vine, "Yaadiyar kada" (Hausa) is locally used for treatment of diabetes but the scientific basis for this has not been established.

1.2 Justification

Diabetes mellitus is a potentially morbid condition with high prevalence worldwide; therefore the disease constitutes a major health concern. Presently, it is an incurable metabolic disorder which affects about 2.8% of the global population. It is one of the chronic diseases that affect both the young and old in our society. According to World Health Organization (2008), at least 171 million people worldwide suffer from diabetes and it is more prevalent in developed countries. According to American Diabetes Association (2008), there were about 20.8 million people with diabetes in United States alone, while in developing countries, increase in prevalence is expected to occur especially in Africa, where most patients will likely be found by 2030. This predicted increase in incidence of diabetes in

developing countries follows the trend of urbanization and lifestyle changes perhaps most importantly a “Western – Style” diet (World Health Organization, 2008).

Pharmaco-therapeuticinterventionsareexpensiveandoftenassociatedwithadverseeffects, hencetheneedforalternativetreatmentmodalities. The hypoglycemic agents currently used in clinical practice have characteristic profiles of adverse side effects (Williams and Pickup, 1991).Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed (WHO, 2002). This is because of the inability of existing modern therapies to control all pathological aspects of the disorder, as well as enormous cost and non-availability of the modern therapies tomany rural populations in developing countries. There is thus a clear need for development of indigenous, inexpensive anti-diabetic agent from plants.

Theincreasinguseofcomplementaryandalternativemedicine(CAM) has arisen as one of such intervention, thus many natural supplements are widely used around the world to treat diabetes. However, only few have scientific proof of efficacy and thus the need to evaluate such natural products in experimental studies.

According to the World Health Organization (WHO), medicinal plants would be the best source of obtaining a variety of drugs. Results of many recent studies have demonstrated the promising potentials of medicinal plants used in various traditional and complementary systems for the treatment of human diseases.

1.3 Aim

The aim of this study is to establish the anti-diabetic potential of the leaf extract of *Tacazzea apiculata*.

1.4 Objectives

1. To determine qualitatively, the secondary metabolites present in the leaf extract of *T. apiculata* by way of phytochemical screening.
2. To determine the effect of *T. apiculata* methanol leaf extract on glucose level in normal and alloxan-induced diabetic rats.
3. To determine the effect of *T. apiculata* methanol leaf extract on lipid profile, haematocrit, body weight and kidney health of alloxan-induced diabetic rats.

1.5 Hypotheses

i. There are no detectable quantities of secondary metabolites and phytochemicals in theleaf extract of *T. apiculata*.

ii. There is no significant antidiabetic activity exhibited by the leaf extract of *T. apiculata***.**

iii. There are no significant effects on lipid profile, haematocrit, body weightand kidney health exerted bythe leaf extract of *T. Apiculata*on alloxan-induced diabetic rats.

CHAPTER TWO
2.0 LITERATURE REVIEW

2.1 Botany and Classification of *Tacazzeaapiculata*

2.1.1 Botany of *Tacazzea apiculata*

Tacazzea apiculata Oliver is a twining woody climber with multiple stems. It is indigenous to tropical Africa (Ahmed*et al*, 2009) which belongs to the family Asclepidiaceae. **(**Bingtao *et al.,* 2014). The leaves are characteristically simple**,** opposite occasionally whorled, very rarely alternate. The inflorescences are terminal and axillary. They have actinomorphic flowers that are usually bisexual.*Tacazzea apiculata* exudes white milky sticky latex.

2.1.2 Classification of *Tacazzea apiculata*

Domain: Eukaryota

Kingdom: Plantae

Subkingdom: Viridaeplantae

Phylum: Magnoliophyta

Subphylum: Spermatophytina

Infraphylum: Angiospermae

Class: Magnoliopsida

Subclass: Lamiidae

Superorder: Gentiananae

Order: Gentianales

Family: *Asclepidiaceae*- asclépiades, milkweeds

Genus: *Tacazzea*

Specific epithet: *apiculata* - Oliver

Botanical name: - *Tacazzea apiculata* Oliver

(Source Bingtao*et al.,* 2014)

2.1.3 Ethnopharmacy of *Tacazzea apiculata*

Tacazzea apiculata Oliv. is popularly called “Craw-craw” vine. The Hausas of northern Nigeria refer to it as “Yaadiyar kada” (Ahmed *et al*., 2009). The powdered leaves are used in snake bite and stings by venomous animals (Burkill, 1997). The flowers are edible. In South Africa, the twig is powdered and taken in milk or water as “tonic” to improve the general health condition of the body. The leaves are used for skin diseases.

2.1.4 Ethnomedicine of *Tacazzea apiculata*

In northern Nigeria the powdered root mixed with milk or honey is taken orally to relief pains in pile. The plant is also reported to be used in traditional medicine for the treatment of some forms of cancers and inflammation. The decoction of the root is used for treating convulsion, epilepsy and as an anti-diabetic by the Hausa people of the North-western Nigeria (personal communications).

It is used in Hausa traditional medicine for the treatment of inflammation (Abubakar *et al*., 2007). Little scientific record is available on the phytochemicals, toxicity and the therapeutic activities of *Tacazzea apiculata.*

2.1.5 Ethnopharmacology of *Tacazzea apiculata*

The acute toxicity studies, analgesic activity and anticonvulsant activities have been demonstrated (Abubakar*et al*., 2007; Ahmed *et al*., 2009). Ahmed *et al* (2009) reported anticonvulsant activities of the fractionated methanol extract of the root using chloroform and n-butanol and reported that the extract had 80 % protection on the chicks from maximal electroshock test an indication of the anticonvulsant activity, though no effect on the mice against penty;enetetrazole-induced seizure.

2.2 Brief History ofDiabetes

Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago. A study of Ayurvedic literatures, written as early as 4th to 5th

century B.C., indicate that diabetes, known as “Madhumeha (honey urine)”, was fairly well known and well-conceived in ancient India. The earliest recorded evidence of their use in classical Indian texts such as Rigveda and Atherveda, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The most authentic medical treatise, Sushruta Samhita, describes 760 species of antidiabetic plants, while Charaka Samhita describes 500 species. They also described plants for glycosuria, polyphagia, and polyuria.

Historical references to diabetes mellitus occur in an ancient Egyptian medicine document, Ebers Papyrus, from the 3rd Dynasty in 1500 B.C. In China, Ben Jing, written in 104 B.C., describes about 252 species of antidiabetic plants. In the Unani system of medicine, which originated in Greece and evolved within the Muslim world, there are various references to diabetes and antidiabetic herbs. South America and Africa, which have less documentation, also described various treatments for diabetes using medicinal plants. A comprehensive review on antidiabetic medicinal plants has been compiled by AttaurRahman and Zaman, providing information regarding nearly 343 antidiabetic plants. The earliest recorded attempt to treat diabetes mellitus dates back more than 3,500 years and the treatment used was of plant origin. Hence, there are various evidences of treatments with antidiabetic medicinal plant in Native American, Traditional Chinese Medicine, Kampo, Ayurvedic, Siddha, Unani, Homeopathy, Tribal, and Folk from time immemorial.

2.3 The Role of Pancreas and Insulin Production in Diabetes Mellitus Causation

The pancreas, in addition to its digestive functions, secretes two important hormones, *insulin* and *glucagon*, that are crucial for normal regulation of glucose, lipid, and protein metabolism (Guyton and Hall, 2006). Anatomically and physiologically, the pancreas is composed of two major types of tissues, the *acini* (which secrete digestive juices into the duodenum) and the *islets of Langerhans* (which secrete insulin and glucagon directly into the blood) (Guyton and Hall, 2006).The human pancreas has 1 to 2 million islets of Langerhans,

each which measures only about 0.3 millimeter in diameter and organized around small capillaries into which its cells secrete their hormones. The islets contain three major types of cells, *alpha*, *beta*, and *delta* cells, which are distinguished from one another by their morphological and staining characteristics (Guyton and Hall, 2006). The beta cells, constitutes about 60 per cent of all the cells of the islets, lie mainly in the middle of each islet and perform its major function ofinsulin secretion (Guyton and Hall, 2006). Changes in insulin secretion or its metabolic pathways results in a metabolic disorder called diabetes.The main purpose of this section of the literature review is to discuss the physiologic roles of insulin and the pathophysiology of the disease, especially *diabetes mellitus*, caused by abnormal secretion or activity of this hormone. Also this literature equally reviewed the treatment, management, draw backs of diabetes mellitus management.

2.4Pathophysiology of Diabetes Mellitus

2.4.1 Description of Diabetes

By description, diabetes is a chronic disorder ora chronic condition that occurs in a metabolic pathway where people do not produce enough insulin to meet their body's needs and/or their cells don't respond properly to insulin produced in the pancreas**.**WHO (2008) defined diabetes asa chronic disease primarily defined by high levels of blood glucose, (hyperglycaemia) giving rise to risk of tiny blood vessel damage (retinopathy, nephropathy and neuropathy). Ozougwu*et al*, (2013) further defined diabetes as a group of metabolic disorders characterized by a chronic hyperglycemic conditions resulting from defects in insulin secretion, insulin action or both. Mercola, (2014) stated that diabetes is disorder of insulin and leptin signaling which occurs when insulin is not available or doesn't work correctly to move glucose from the blood into cells, glucose will stay in the blood and high blood glucose levels are toxic. It is pertinent to state therefore, that when cells that do not

get adequate supply of glucose are lacking the fuel they need to function, they will eventually malfunction leading changes in the entire body equilibrium.Insulin is important secretion of the pancreas, because it moves glucose, a simple sugar, into the body's cells from the blood. It also has a number of other effects on metabolism. Insulin acts as a key chemical message transmission that tells the cell to open and receive glucose (Guyton and Hall, 2006).

Diabetes mellitus is a chronic disease with an increasing prevalence worldwide (Akinkugbe, 1997), which is now ranked the sixth leading cause of death worldwide (MMWR, 2002). It constitutes a significant health and socioeconomic burden for patients and the health care systems. American diabetic's statistics (2011) echoed that diabetes has increased more than 700 percent in the last 50 years. According to the WHO, there were 150 million diabetic patients worldwide by the year 2000, with a projection of 221 million people in 2010 and 300 million in 2025 (Akinkugbe, 1997). The International Diabetes Federation (IDF) estimates that over 5 million people suffer from the disease in Africa and the number is expected to increase to 15 million by 2025 (Akinkugbe, 1997).

The diabetes prevalence in Nigeria varies from 0.65% in rural Mangu village to 11.0% in urban Lagos (Nathan *et al.,* 2005). The incidence of diabetes in the African population is steadily on the rise and this translates to increase the incidence of late diabetic complications (Wild *et al* 2004). However these eventually pose major health care and economic problems. The WHO suggests that Nigeria has the greatest number of people living with diabetes in Africa, with an estimated burden of about 1.7 million which will increase to 4.8 million by 2030. The economic burden of diabetes is enormous in terms of the direct cost of intensive monitoring and control of blood glucose and managing cardiovascular, renal, and neurological consequences (Zimmet, 2003).

2.4.2 Risk factors in diabetes

According to the National Institutes of Health, the following are major risk factors for diabetes and pre-diabetes:Age 45 or older, family history of diabetes, overweight, inactive lifestyle (exercise less than 3 times a week), African-American, Hispanic/Latin American, American Indian and Alaska Native, Asian-American, or Pacific Islander ethnicity, high blood pressure (140/90 mm Hg or higher), HDL (good) cholesterol less than 35 mg/dL or triglyceride level 250 mg/dL or higher, have had diabetes during pregnancy (gestational diabetes) or have given birth to a baby that weighed more than 9 pounds, Polycystic ovary syndrome (metabolic disorder that affects female reproductive system), Acanthosis nigricans (dark, thickened skin around neck or armpits), history of disease of blood vessels to the heart, brain, or legs, diabetes test history of impaired fasting glucose (IFG) or impaired glucose tolerance (IGT).

2.4.3 Medical conditions associated with increased risk of diabetes

2.4.3.1 *Obesity and Metabolic Syndrome*

Obesity is the number one risk factor for type 2 diabetes. Excess body fat appears to play a strong role in insulin resistance, but the way the fat is distributed is also significant. Weight concentrated around the abdomen and in the upper part of the body (apple-shaped) is associated with insulin resistance and diabetes, heart disease, high blood pressure, stroke, and unhealthy cholesterol levels. Waist circumferences greater than 35 inches in women and 40 inches in men have been specifically associated with a greater risk for heart disease and diabetes. (People with a "pear-shape" -- fat that settles around the hips and flank -- appear to have a lower risk for these conditions.) However, obesity does not explain all cases of type 2 diabetes. It is also common among people in countries where weights tend to be low, such as Asia or India.

A set of conditions referred to as metabolic syndrome (also called Syndrome X) is a pre-diabetic condition that is significantly associated with heart disease and higher mortality rates from all causes. The syndrome consists of obesity marked by abdominal fat, unhealthy cholesterol and triglyceride levels, high blood pressure, and insulin resistance.

2.4.3.2 *Polycystic Ovary Syndrome*

Polycystic ovary syndrome (PCOS) is a condition that affects about 6% of women and results in the ovarian production of high amounts of androgens (male hormones), particularly testosterone. Women with PCOS are at higher risk for insulin resistance, and about half of PCOS patients also have diabetes.

2.4.3.3 *Depression*

Severe clinical depression may modestly increase the risk for type 2 diabetes.

2.4.3.4 *Schizophrenia*

While no definitive association has been established, research has suggested an increased background risk of diabetes among people with schizophrenia. In addition, many of the new generation of antipsychotic medications may elevate blood glucose levels. Patients taking antipsychotic medications (such as clozapine, olanzapine, risperidone, aripiprazole, quetiapine fumarate, ziprasidone) should receive a baseline blood glucose level test and be monitored for any increases during therapy.

2.5Types and Causes of Diabetes

Scientific studies have identified three major types of diabetes namely: type 1, type 2 and gestational diabetes. Each of these types is typically associated with unique circumstances.

2.5.1 Type 1 diabetes

Type 1 diabetes is equally called “insulin dependent diabetes” or “juvenile onset diabetes” This type of diabetes accounts for 5-10 percent of people with diabetes and it is associated with infants of 4 to 5 years or with teens and early adulthood (Blood *et al*., 1975). The incidence of type 1 diabetes is alarming, across Europe, the incidence among children under 15 years is 3.4 % (EURODIABACE study group, 2000) with the steepest rise in those under 5 years old (Karvonen*et al*, 1999).

Type 1 diabetes can be as a result of autoimmune reaction to proteins of the islets of Langerhans in the pancreas (Holt, 2004). This occurs when there is an immune disorder which may be due to auto immune reaction leading to autoimmune destruction of beta cells(insulin producing cells in the pancreas) resulting to a loss of the hormone insulin (Mercola, 2014). According to southern cross medical library (2012) the cause of autoimmune reaction or destruction of the beta cells are not known but have been postulated to be caused by certain virus infection or environmental factors as well as genetic factors are the major causes of the disorder. Type 1 diabetes is clinically characterized by low to normal body mass, low or no plasma insulin, high plasma glucagon that can be suppressed, increased plasma glucose, normal insulin sensitivity, and usually associated with infants (Guyton and Hall 2006).These autoimmune reactions are usually characterized by three auto-antibodies which includes;

Islets cell surface autoantibody (ICCA). The ICCA is primarily an indicator for accurate predictor of future development of type 1 diabetes and this accounts for 90% of type 1 diabetes that are against islets cells cytoplasmic proteins.

Islets cells surface autoantibody (ICSA). ICSA is an autoantibody directed to islets cell surface antigens and this may cause 80% type 1 diabetes as it has been identified with 80% patients

with type 1 diabetes.Specific Antigenic targets of islets cells. Antibodies to glutamic acid decarboxylases (GAD) and this has been identified with 80% of type 1 diabetics and little in some type 2 diabetics. The onset of clinical symptoms of type 1 diabetes represents the end stage of beta cells destructions (Ozogwu*et al*., 2013) and the characteristic were highlighted by Al Homsi and Lukic (1992) as follows:

Presence of immune-competent accessory cells in the infiltrated pancreatic islets.Associations of susceptibility to disease with class II genes of the major histocompatibility complex associated with immune response.Presence of islets cells specific auto antibodies**.** Alterations of T cells mediated immunoregulations**.** The involvement of monokines and TH1 cells producing interleukins in the disease process.

Response to immunotherapy and finally frequent occurrence of other organ specific auto-immune disease in affected individuals or in their family members. (Ozougwu*et al*, 2013).

2.5.2 Type 2 diabetes

Type 2 diabetes is a predominant form of diabetes and accounts for atleast 90 % of total diabetes mellitus cases (Gonzalez *et al* 2009) also called non-insulin dependent diabetes mellitus is caused by decreased sensitivity of target tissue to insulin often called insulin resistance and its causes includes; obesity/overweight, excess glucorticoids, excess growth hormones, pregnancy and gestational diabetes, polycystic ovary disease, lipodystrophy, autoantibodies to insulin receptor, mutations of insulin receptor, mutations of the peroxisome proliferators activator receptor, mutation that causes genetic obesity like melanocortin receptor mutations and finally hemochromatosis which is a hereditary diseases that causes tissue iron accumulation(Guyton and Hall, 2006; Kaku, 2010; Ozougwu *et al*., 2013).When the cells are unable to use insulin properly, particularly by the cells in the liver and this is commonly called “adult-onset diabetes” or non-insulin dependent diabetes

since it is diagnosed later in life. Type 2 diabetes is a heterogeneous disorder caused by a combination of factors, hencemultifactorial diseases involving multiple genes and environmental factors to a varying extent (Holt, 2004).

The basic effect of insulin lack or insulin resistance on glucose metabolism is to prevent the efficient uptake and utilization of glucose metabolism by most cells of the body except the brain (Guyton and Hall, 2006) and this results to increase in blood glucose concentrations and sharp decrease or falls in cell utilization of glucose and increase in utilizations of fat and protein (Ozougwu *et al*., 2013).Type 2 diabetes is clinically characterized by obsessed body mass, normal to high plasma insulin at the initial time, high plasma glucagon that is resistant to suppression, increased plasma glucose and reduced insulin sensitivity (Guyton and Hall, 2006). The insulin resistance preceding type 2 diabetes is commonly referred to as the metabolic syndrome (Yki-Jarvinen H.,2011) and this condition consists of a cluster of risk factors, which are thought to be either causes or consequences of insulin resistance. The development of type 2 diabetes, overt hyperglycaemia, also requires the presence of a relative defect in insulin secretion. This defect appears, at least in part, genetically determined hence, the causes of type 2 diabetes have been attributed to hereditary factor, aging and obesity (Yki-Jarvinen H. 2011; Southern Cross Medical Library, 2012).

It is thought that as people age, beta cells become less efficient and the cells in the body become less able to use the insulin made by the pancreas. The type 2 diabetes is caused by a combination of genetic factors related to impaired insulin secretion, insulin resistance and environmental factors like obesity, overeating, lack of exercise, aging and stress. However, hepatic insulin resistance and metabolic syndrome can be linked to atherosclerosis and cardiovascular disease, the main cause of the excess mortality in type 2 diabetes, by increased very-low-density lipoprotein production which leads to the generation of small, dense, and atherogenic LDL particles (Yki-Jarvinen H., 2011) . Insulin resistance is also seen in

adipose tissue and skeletal muscle, altering glucose and fatty acid handling in these tissues and the liver. The hepatic manifestation of insulin resistance in type 2 diabetes is nonalcoholic fatty liver disease (NAFLD) (Yki-Jarvinen H., 2011). NFALD is defined as excess fat in the liver which is not due to excess alcohol use, and can lead to hepatic inflammation and even cirrhosis. It can be rightly deduced that that type 2 diabetes is caused by insulin and glucagon defects. Type 2 diabetes is associated with some risk factors such as obesity, insufficient energy consumption, alcohol drinking, smoking and aging (Ozougwu *et al*.,2013).

2.5.3 Gestational diabetes

This is an uncommon form of diabetes as the name implies, that occur during pregnancy accounting for approximately 2 - 4% of cases in pregnant women (Southern Cross Medical Library, 2012).However, the condition usually disappears after the pregnancy. Gestation diabetes manifest as either Type 1 or Type 2 diabetes. Gestational diabetes is thought to occur as a result of hormonal changes during pregnancy in women who are genetically predisposed to develop diabetes

Other causes ofdiabetes include:

Medications (eg: prednisone a steroid medication) and some diuretics (water tablets), which can increase the chances of developing diabetes by increasing insulin resistance.

Certain medical conditions - eg: diseases of the pancreas (eg: chronic pancreatitis) and some disorders of the hormone-producing endocrine glands (eg: Cushings' syndrome).

Trauma or surgery - eg: injury to the pancreas or surgical removal of the pancreas.

Some genetic disorders eg: Down's syndrome, Turner's syndrome and some muscular dystrophies

2.6Signs and Symptoms of Diabetes

The onset of symptoms in Type 1 diabetes is typically quite sudden and symptoms can be severe. However, the symptoms of Type 2 diabetes tend to manifest gradually, so much so that they may go unnoticed (Mercola, 2012). When hyperglycaemia (excess glucose in the body) occurs, the body tries to get rid of the excess glucose by excreting it in the urine (Guyton and Hall, 2006). This increases urine output and can lead to dehydration. At the same time the body's cells are starved of the glucose energy they need. Common symptoms of diabetes according to Southern Cross Library (2012) include: extreme thirst, frequent urination, blurry vision, extreme hunger, increased tiredness/ fatigue, unusual weight loss, irritability, unusual weight gain or loss, slow healing of wounds, numbness in arms and feet and frequent infections in the skin, vaginal and urinary.

The signs and symptoms of diabetes are always accompanied by a variety of complications which may be acute and chronic in activities.

2.6.1 Diabetic complications

Patients with diabetes have higher death rates than people who do not have diabetes regardless of sex, age, or other factors. Heart disease and stroke are the leading causes of death in these patients. All lifestyle and medical efforts should be made to reduce the risk for these conditions.

People with type 2 diabetes are also at risk of nerve damage (neuropathy) and abnormalities in both small and large blood vessels (vascular injuries) that occur as part of the diabetic disease process. Such abnormalities produce complications over time in many organs and structures in the body. Although these complications tend to be more serious in type 1 diabetes, they still are of concern in type 2 diabetes. Most people with diabetes should aim

for fasting blood glucose levels of less than 110 mg/dL and hemoglobin HbA1C of less than 7%.

There are two important approaches to preventing complications from diabetes:

Intensive control of blood glucose and keeping glycosylated hemoglobin (HbA1c) levels below 7%. Tight blood glucose and HbA1c control can help prevent complications due to vascular (blood vessel) abnormalities and nerve damage (neuropathy) that can cause major damage to organs, including the eyes, kidneys, and possibly the heart.

Managing risk factors for heart disease. Control of blood glucose also helps the heart, but its benefits occur over time. It is very important that people with diabetes control blood pressure, cholesterol levels, and other factors associated with heart disease. (IDF, 2009).

Heart disease

Kidney damage (nephropathy)

Nerve disorders (neuropathy)

Foot ulcers and amputations

Charcot Foot

Retinopathy and eye complications

Mental function and dementia

Infections

Depression

Hypoglycemia

Diabetic ketoacidosis (DKA)

Other complications

Specific complications in women (IDF, 2009).

2.7 Diagnosis of Diabetes

Early diagnosis and treatment is very vital to treatment and management of diabetic related complications. Current diagnosis involves the use of blood samples to ascertain the blood glucose level. The diagnosis of diabetes is well known and this includes the following methods (Southern Cross Library, 2012): fasting blood glucose test, Random blood glucose test, HbA1ctest.

2.7.1 Fasting blood glucose test

A sample of blood is taken to measure the blood glucose levels in the blood after the person has not eaten for several hours (Mercola, 2014). This is usually performed in the morning, before breakfast. It is the most common blood test used to assist with diagnosis.

2.7.2 Random blood glucose test

A sample of blood is taken to measure the glucose levels in the blood, regardless of when the person last ate (Mercola, 2014). The acceptable range for glucose in the blood is 4.0 - 8.0mmol/L (mmol of glucose per litre of blood) before a meal. A diagnosis of diabetes can usually be made if there are classical symptoms of the condition and blood glucose levels of greater than 11mmol/L for a random blood glucose test, or greater than 7mmol for a fasting blood glucose test.

2.7.3 HbA1c test

A blood test called the haemoglobin A1c (HbA1c) assay is often used to indicate a person's average blood glucose levels over a period of time, and this test is useful in monitoring the progress of the condition and the effectiveness of treatment (Mercola, 2014). To further confirm the diagnosis of diabetes, a glucose tolerance test (GTT) may be recommended. This is a very sensitive test in which the person drinks a measured quantity of glucose after having a sample of blood taken. Further blood samples are taken at fixed intervals in the two hours following the glucose drink. The results will indicate how the body responds to increased glucose levels in the blood. In a person who does not have diabetes, the blood glucose levels will rise after the drink, then slowly fall as insulin is produced to metabolise it. In a person with diabetes, the levels of glucose in the blood will remain high for a long time, or they may be very slow to fall.

Urine tests may also be done and will demonstrate high levels of glucose. They are not used in isolation in the diagnosis of diabetes, as the results can be misleading. Other diagnostic tools involve a routine exam by an eye, the kidney function and the heart doctor or foot doctor will reveal diabetes. Diabetes affects the circulation to your feet and the tiny blood vessels in your eyes.

2.8Management and Treatmentof Diabetes

It is well established that good glycaemic control and control of other cardiovascular risks in diabetic patients lead to reduced morbidity and mortality (Nathan *et al*., 2003). Several studies, like the Diabetes Complications and Control Trial (DCCT) etc found that the adequate management of diabetes improves the outcome in terms of morbidity, mortality, and quality of life (Schram *et al.,* 2003). For this reason, it is necessary to follow recommended international and national guidelines in managing diabetic patients (IDF, 2006).Management practices includes: glycaemic targets, systolic and diastolic blood pressure targets, body mass index (BMI) and other anthropometric indices, lipid levels, self-

management, and behavioral targets. More so, Okolie (2006) documented and stated that diabetes education is critical for ensuring the attainment of targets in patients under treatment.

Diabetes and its complications have huge disease burdens globally especially in developing countries and its management in Nigeria is highly suboptimal. However, while diabetes cannot be cured, it can be controlled. Most treatment strategy is targeted at maintenance of equilibrium in the blood glucose level usually between 4.0 and 8.0 mmol/L (Southern Cross Library, 2012) hence life style changes is very vital. Effective control of diabetes requires strict and routine examination by a medical professional, diabetic education, monitoring of the patients and therapeutic treatment of the disease (WHO, 2009). Health promotion, early detection, knowledge of the condition and effective monitoring of blood glucose levels are also considered to be important in effective management of diabetes hence the need for a guideline for the management and treatment of diabetes (Nwankwo *et al.,* 2010).

Effective treatment of Diabetes involves two stipulated guideline which involves the following:

life style changes andmedications

2.8.1 Life style changes

Life style factors includediet regulations, and regular exercise. The diet entails avoidance of food that is rich in refined sugar like fructose (Mercola, 2014), such as pastries, chocolate, juices, sweet biscuit etc. Consumption of food rich in natural carbohydrates which can be readily converted to glucose and this includes cereals, vegetables and fruits. More so, diet should be low in fat but high in dietary fiber, with reduction or abstinence from alcohol consumptions. Also having a stable eating pattern is recommended as a strategy to maintain

glucose equilibrium. Diabetes patients are advised to consume plenty Omega-3 fats from a high quality animal based sources and equally probiotics should be regularly consumed.

Regular but moderate exercise is important in maintaining balanced blood glucose levels (Mercola, 2014). Exercise also helps to maintain a healthy body weight and control high blood pressure and high blood cholesterol levels. This in turn helps to reduce the risk of related health conditions such as cardio-vascular disease (heart attacks and strokes).

Other life style changes includes stress management, it is believed that when the body is stressed, the blood glucose levels are more difficult to control. It is therefore important that stress is adequately managed. It is recommended that people with diabetes do not smoke. Smoking plays a part in increasing the risk of developing many diabetic complications. Illness can also make blood glucose levels more difficult to control. If blood glucose levels become unstable due to illness, it is important to consult a doctor - particularly if vomiting occurs.

2.8.2 Medications

Because diabetes is a disorder of insulin deficiency or malfunction the cure for diabetes requires metabolic pathway that utilizes and activates insulin activities in the blood (Guyton and Hall, 2006). People with Type 2 and type 1 diabetes may not be able to adequately control their blood glucose levels through diet, exercise and lifestyle changes alone but need to take Insulin injection.

Therefore, in many cases diabetic tablets are required. Tablets have different modes of actions and this includes (Ozougwu *et al.,* 2013):

Tablets to increase insulin output from the pancreas eg: Glipizide, Glibenclamide. Tablets to reduce insulin resistance eg: Metformin. Tablets that stop the absorption of carbohydrates from the bowel eg: Acarbose.

Insulin injections and tablets available for the treatment of diabetes varied based on duration of the effectiveness of the insulin drug. While some insulin are short-acting meaning they are absorbed quickly by the body and are effective for a short period of time, others are long-acting; meaning they are absorbed more slowly by the body and are effective for a longer period of time. Both short term and long term combination are usually used and the amount of insulin required, and how often it is to be administered will vary according to the needs of the individual and the glucose levels in the blood. Usually blood glucose levels are tested, finger prick test and is measured on a special testing strip. The results will help to determine how much medication is required, how much exercise can be undertaken and what foods should be eaten.

2.9Alternative Treatment and Research

While no cure for diabetes is known, research is continuing in order to develop new and more effective management and treatments for the condition. Some of the areas being researched include:

Alternative ways of delivering insulin that do not involve injections eg: nasal sprays, patches and tablets.

Devices that can measure blood glucose levels through the skin rather than having to obtain a finger prick of blood.

Transplantation of insulin-producing pig-cells into humans. This research is controversial and is at an early stage.

Bio-engineering techniques that may allow researchers to create artificial insulin-producing cells.

The use of plant based drugs either as combination therapy or as lone single drugs for the treatment of diabetes and other diabetes related complications.

Other promising areas of research for treatment of diabetes involve the gene therapy technique which involves the genetic manipulations of the tissue or cells in vivo or ex-vivo. The genetic manipulations involves identification of the potential sources of new beta cells, replacement therapy, expression of gene encoding insulin and glucose lowering hormones in the recipients own cells (Shaw and Doherthy, 2011).

Since both type 1 and type 2 diabetes are associated with decline in beta cells mass, cell and gene therapy targeted at beta cell and insulin replacement proves a good potential forms of diabetes treatment (Shaw and Doherthy, 2011).

2.9.1 Phytomedicinal antidiabeticpharmaceuticals

Nowadays, insulin and other oral blood glucose lowering agents are used in the clinical management of diabetes mellitus. Unfortunately, the prevalence of this disease continues to rise worldwide and little can be done to prevent the delay of its secondary complications. Thus, the search for new antidiabetic drugs with novel mechanisms of action should still be pursued. Man has used plants heavily to treat diabetes mellitus, so much so, there are 700 recipes containing more than 400 plants reputed for their antidiabetic activity. The last few years have seen a major surge in the use of herbal medicines the world over. Africa is sitting on a gold mine of well-recorded and well-practiced knowledge of traditional herbal medicine, and hence must capitalize on this herbal wealth by promoting its use worldwide.

In the field of bioinformatics, molecular docking studies are widely used to predict suitable drug candidates in the pharmaceutical drug designing industry. Binding orientation of these as possible drug candidates.

Plant based drugs or system of traditional medicine according to WHO is still the mainstay of about 75 to 80% of the world population, mainly in the developing countries for primary

health care because of the cultural acceptability better compatibility with human body and lesser side effects (Central Council for Research in Ayurveda and Siddha, 2008). Recent molecular investigations all over the world highlight the power of herbs and its increased awareness has led to the renewed interest in herbal medicines across the world, in recent times and thus plant based drugs has been viewed as the power house sources for antidiabetic drugs (Saravanamuttu and Surdarsanam, 2012).

The earliest recorded attempt to treat diabetes mellitus dates back to more than 3,500 years and the treatment was used was of plant origin (Swanston-Flatt *et al*., 1989), however, Saravanamuttu and Surdarsanam, 2012 noted that herbs were used to treat diabetes before the advent of insulin injections. It is pertinent to state that in most recent times, research in herbal remedy for diabetes has increased because it is believed that a wide array of chemically derived compound with antidiabetic propertyfrom plants are relatively safe.

Antidiabetic plants have been listed to belong to the family Leguminoseae (Fabaceae), Curcubitaceae, Laminaceae, Liliaceae, Asteraceae, Rosaceae, Euphorbiaceae, Moraceae, and Araliaceae (Saravanamuttu and Surdarsanam, 2012).

Although a number of plants have shown some degree of antidiabetic activity, a significant amount of research as well as traditional usage is confined only to few useful plants (Saravanamuttu and Surdarsanam, 2012), some of which are the following: *Gymnema sylvestre* (Periploca of the woods or Rams' horn), *Trigonella foenum-graecum* (Fenugreek or Sicklefruit fenugreek), *Momordica charantia* (bitter gourd), *Opuntia streptacantha* (nopal or prickly pear cactus), *Pterocarpus marsupium* (Indian Kino Tree or Malabar Kino Tree), *Polygala senega* (Seneca snake-root, senegaroot, milkwort, rattlesnake root or mountain flax), *Allium sativum* (garlic), *Panax quinquefolius* (ginseng, dwarf groundnut or root of life), *Aloe vera* (Aloe or First Aid Plant), *Lagerstroemia speciosa* (banaba tree or pride of India),

Tinospora cordifolia (Heart-Leaved Moonseed or Heavenly Elixir), *Syzygium cumini* (black plum or jambolan), *Azadirachta indica* (neem),*Murraya koenigii* (curry leaf tree), *Emblica officinalis*(Indian gooseberry or amla), *Phyllanthus amarus* (stone breaker or sleeping plant), *Cyamopsis tetragonoloba* (guar bean, cluster bean or guar gum tree), *Withania somnifera* (winter cherry or poison gooseberry), *Hordeum vulgare* (barley), *Ginkgo biloba* (ginkgo or maidenhair tree), *Bauhinia forficata*(Brazilian orchid tree), *Ocimum sanctum* (holy basil), Coccinia *indica* (baby watermelon or ivy gourd), *Vaccinium myrtillus* (bilberry) and G*lycyrrhiza glabra* (licorice).

Studies with*Pterocarpus marsupium* (ICMR Study group, 2005) and *Bauhinia forficata* (De Sousa *etal.,* 2004) revealed that both have the ability to promote regeneration of the damaged beta cells in the Islet of Langerhans in the pancreas. There is also some scientific prove that some plant have blood glucose lowering activity and some in addition have antioxidant as well as hypocholesterolemic activities (Saravanamuttu and Surdarsanam, 2012). While numerous antidiabetic effect has been identified and the active ingredient isolated, in plants little is known about some plants that are used as antidiabetic agents in some community. It is believed that presence and isolation certain active ingredient have shown mechanistic reduction of glucose level in the blood and according to Saravanamuttu and Surdarsanam (2012) the active ingredients play a vital role in enhancing glucose utilization, lowering plasma glucose and improving insulin sensitivity in diabetic animals.

Results from numerous studies has shown that plant extract has shown hypoglycemic effects when compared to glibenclamide, tolbutamide and metformin as standards (Howlett and Bailey, 2007; Alexious and Demopoulus, 2010; Bhowmik *et al*., 2011).

Active ingredients from *Abies pindrow* (silver fir) and its active ingredients D-pinitol, exerts an insulin-like effect to improve glycemic control in hypoinsulinemic STZ-diabetics mice and D-pinitol may act via a post-receptor pathway of insulin action affecting glucose uptake

(Saravanamuttu and Surdarsanam, 2012). Equally hypoglycemic activities similar to the activity of insulin were seen in some plants on experimentally induced diabetes in rats. *Bauhania purperea* was reported to have anti-hyperlipidemic and anti-oxidant activity in addition to antidiabetic acitivity (Muralikrishna *et al*, 2008).

Certain plants have been reported to have enhancing effect on the beta cells thereby promoting their insulin glucose activities which in turn have antidiabetic potential. Saponins of root back of *Barberis vulgaris* aqueous extract exhibited significant anti-hyperglycemic activities which can be attributed to its ability to stimulate the proliferation and regeneration of remnant beta cells (Upwar *et al*., 2011). Equally root of *Beta vulgaris* is known to reduce blood glucose levels by regenerations of beta cells. Atmakuri and Dathi (2010) reported hypoglycemic activities of *Biophytum sensitivum*leaf extract mediated throughstimulating the synthesis/ release of insulin from the beta cells of langerhans.

Antidiabetic property were seen in *Eucalyptus globules* (Chauhan *et al.,* 2010) and *Bougainvillea spectabilis*(Adebayo, 2005) (methanol extract) extract due to their potential to repair damaged beta cells and their ability to restore impaired pancreatic beta cells.

Active ingredients or phytochemicals which are secondary metabolites have been isolated from plants and the hypoglycemic constituent includes alkaloids, flavonoids, triterpenoids, polysaccharides, glycopeptides, amino butyric acid derivatives, steroids, iridoids, phenolics, peptides, alkyl disulfides and inorganic ions (Bever and Zahnd, 1979).

Natural products in the form of phytochemicals play vital roles in enhancing glucose utilization, lowering plasma glucose and improving sensitivity in diabetic studies using animal models and therefore can provide important clues for identification and synthesizing novel drugs with antidiabetic potentials. However, it is important to study the toxicity, therapeutic efficacy, shelf life and stability of plant based herbal medicines that are traditionally used for treatment of diabetes mellitus.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Materials

Fresh leaves of *Tacazzea apiculata* were collected from the wild around Zaria Dam, Zaria in the month of May 2014. They were taken to the herbarium of the department of Biological Sciences in A.B.U. Zaria for taxonomic authentication. A voucher specimen number 6975 matched with the collected plant. The leaves of *Tacazzea apiculata* were then air-dried at room temperature and then pounded to powder using pestle and mortar.

3.2 Experimental Animals

A total of forty three (43) male Wister albino rats of body weight range 160-250g were obtained from the departments of Biological Sciences and Veterinary medicine breeding houses in A.B.U. Zaria. The animals were then taken to the animal house of the Department of Pharmacology and Therapeutics of faculty of Pharmaceutical Sciences. They were acclimatized for three weeks in well-aerated cages kept in well-ventilated rooms. During this period, the animals were maintained on standard animal feed and drinking water *ad libitum.* Research procedures were carried out in compliance with conventions.

3.3 Chemicals, Reagents and Glucometers

The drug used for the induction of experimental diabetes, Alloxan Monohydrate was ordered from Sigma Aldrich Chemical Company, St. Louis Mo, U.S.A. The digital glucometer

used for monitoring blood glucose-Accu-Chek Active, Roche Diagnostics, Germany) was obtained commercially from medical equipment suppliers. All other chemicals and reagents such as Sulphuric Acid, Sodium Hydroxide, Ferric Chloride, Mayers' Reagent, Trichloroacetic Acid, Bromine Water and Test kits were commercially obtained and were of analytical grade.

3.4 Plant Extract

The powdered leaves of *Tacazzea apiculata* was subjected to the following Extraction process and Test procedures.

3.4.1 Methanolic extraction

One kilogram (1kg) of the powdered leaves of *Tacazzea apiculata* was macerated in 5L of 70% methanol for 72 hours with frequent shaking. The mixture was thereafter filtered and the filtrate was evaporated to dryness on a regulated hot plate and the residue (extract) was kept until needed (Njike et al, 2005).

3.4.2 Phytochemical screening

The methanolic leaf extract of *Tacazzea apiculata* (MLETA) was subjected to a series of preliminary phytochemical tests to determine the presence or absence of phytochemicals or secondary metabolites, through the following methods.

Three grams (3g) of the MLETA was first dissolved in distilled water and then filtered, the clear filtrate was used for the phytochemical screening, as follows;

3.4.2.1 *Detection of carbohydrates- Molisch's test*

To 2ml of MLETA, a few drops of alcoholic solution of α- naphthol (Molisch reagent) was added, the mixture is shaken well and 1 ml concentrated sulphuric acid is added slowly added along the sides of the test tube to form a lower layer and allowed to stand. A violet or reddish-brown ring at the interface indicates the presence of carbohydrates.

3.4.2.2*Detection of unsaturated steroids and triterpenes, (Liebermann-Burchard's test)*

To 2ml of MLETA, equal volume of acetic acid anhydride was added and mixed gently. To this, 1-2 drops of concentrated sulphuric acid is added slowly along the sides of the test tube to form a lower layer. Colour changes were observed immediately and over a period of one hour. Blue to blue-green colour in the upper layer and a reddish, pink or purple colour indicate the presence of triterpenes (Evans, 1996).

3.4.2.3*Detection of Free Anthracene Derivatives / Anthraquinones (Borntrager's Test***)**

To 2ml of MLETA in a dry test tube, 5ml of chloroform was added and shaken for 5 minutes. It was then filtered and the filtrate was shaken with equal volume of 10% ammonia solution is added to it. A bright pink colour in the aqueous upper layer indicates the presence of freeanthraquinones. (Evans, 1996).

3.4.2.4*Detection of saponins (Frothing Test)*

Ten ml(10ml) of distilled water was added to 2ml of MLETA and shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 minutes. A honeycomb froth that persists for 10-15 minutes indicates the presence of Saponins (Evans, 1996).

3.4.2.5 *Detection of flavonoids (Sodium Hydroxide Test)*

To 2ml of MLETA, a few drops of 10% sodium hydroxide were added to the extract. A yellow coloration indicates the presence of flavonoids (Evans, 1996).

3.4.2.6*Detection of tannins (Ferric Chloride Test)*

To 2ml of MLETA, 3-5 drops of ferric chloride solution was added. A greenish – black precipitate indicates the presence of condensed tannins while hydrolysable tannins give blue or brownish-blue precipitate (Evans, 1996).

3.4.2.7*Detection of alkaloids(Mayer's test)*

To 2ml of MLETA, a drop or two of Mayer's reagent were added by the side of the test tube. A white or creamy precipitate indicates the presence of alkaloids (Evans, 1996).

*Dragendoff's Test***;** to 2ml of MLETA, few drops of Dragendoff reagent were added. A reddish brown precipitate indicates the presence of Alkaloids (Evans, 1996).

3.4.3 Acute toxicity

The lethal dose LD_{50} of the methanolic leaf extracts of *Tacazzea apiculata* was determined as described by (Lorke,1983). In the first phase, nine rats were divided into three groups of three rats each. The first group received the extract dose of 1000mg/kg b.w. the second group received a dose of 100mg/kg b.w. of the plant extract, while the third group received 10mg/kg b.w. of the same extract. The animals were observed for the signs and symptoms of toxicity, including mortality over a period of 24 hours.

Based on the results of the first phase, in the second phase, four rats were divided into four groups of one rat each. The extract was administered at the dose of 1200, 1600, 2900 and 5000mg/kg b.w. to group 1, 2, 3 and 4 respectively. The four animals in the second phase were also observed for signs of toxicity, including death.

The LD_{50} was then calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose, i.e. the geometric mean of the consecutive doses of which 0 and 100% survival rates were recorded (Lorke, 1983).

3.5 Induction of Experimental Diabetes

Diabetes mellitus was induced in a group of normoglycaemic male wistar albino rats, starved for 12 hours. Each rat received a single intraperitoneal injection of 150mg/kg b.w of Alloxan monohydrate, reconstituted in normal saline (Sharma et al, 2010). After 72 hours, each rat's blood glucose level was tested by drawing blood from the tail vein, usinga digital glucometer (Accu Chek Active, Roche, U.S.A.). All rats whose blood glucose levels were greater than 250mg/dL were considered induced and thus hyperglycaemic (Sharma, 2010) and included in the study.

3.6 Treatment of Rats with Plant Extract and Standard Drug (Glibenclamide).

A total of 43 rats were used in this experiment, 13 for the lethal dose (LD_{50}) tests and the other 30 were divided into 6 groups of 5 rats each for the study. The groupings were as follows;

Group A - Normal control, received no treatment.

Group B- Diabetic control, induced but untreated, received distilled water only.

Group C- Diabetic rats, treated with 100mg/kg b.w. dose of*T. apiculata* extract.

Group D- Diabetic rats, treated with 200mg/kg b.w. dose of *T. apiculata* extract.

Group E- Diabetic rats, treated with 400mg/kg b.w. dose of *T. apiculata* extract.

Group F- Diabetic rats, treated with glibenclamide 5mg/kg b.w and served as positive control (Sharma, 2010).

Oral administration of the plant extracts to the diabetic groups C, D and E and oral administration of the standard drug(glibenclamide) to group F started the same day for a period of four weeks. During the four weeks period, rats in all groups were fed with standard

diet and water. Blood samples were drawn from the tail vein for the blood glucose level determination. All rats were weighed prior to the commencement of treatment and subsequently re-weighed every week till the end of the four weeks experimental period. On the last day of the experiment, animals were fasted over-night and blood samples were collected by cardiac puncture under mild ether anaesthesia, for the biochemical estimations.

The standard drug, glibenclamide was commercially obtained and dissolved in distilled water, then orally administered to the group F diabetic rats (positive control), according to their body weights.

3.7 Blood Glucose Level and Body Weight

The body weights of the rats were measured weekly, using a top loader weighing balance. Blood samples were drawn from the tail vein of the rats for blood glucose level determination using a digital glucometer (Accu-chek Active, Roche, U.S.A.), at 0,3,6,9,12 and 24 hours, after treatment with the extract and standard drug, then subsequently at 1^{st}, 2^{nd}, 3^{rd} and 4^{th} week of treatment.

3.8 Packed Cell Volume (PCV)

The PCV was determined using heparinized capillary tubes after the four weeks treatment. With a pair of sterile scissors, the rats were bled by a slide cut on the tail tip. The blood was then collected into heparinized capillary tubes and centrifuged at 3000rpm for 10 minutes. The PCV was then determined using the Hawkley Micro-haematocrit reader.

3.9 Biochemical Estimations

The serum levels of triglyceride (TGL), total cholesterol (TC) and high-density lipoprotein-cholesterol (HDLC) were determined spectrophotometrically, using enzymatic calorimetric assay kits (Randox, Northern Ireland), while the serum levels of low-density lipoprotein

cholesterol, (LDLC), very low-density lipoprotein cholesterol (VLDLC) and antiartherogenic index (AAI) were determined by calculations.

Blood samples were collected from overnight fasted rats under mild anaesthesia on the last day of the experiment. Five ml(5ml) of blood was collected from each rat into ice-cold centrifuge tubes. The blood samples were then centrifuged in a Denley BS400 centrifuge (England) at 5000rpm for 5 minutes. The supernatant (serum) was collected and assayed for the levels of TGL, TC and HDLC, using the Randox Biochemical kits, while the serum levels of LDLC, VLDLC and AAI were derived by calculations.

3.9.1 Serum total triglycerides assay

The serum level TGL was determined using the method as described by Tietz (1990). 1000µl of the reagent was added to 10µl each of the samples and a standard. This was incubated for 10 minutes at 20-25ºC and the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was then measured against the reagent blank within 30 minutes.

$$\boldsymbol{TGL\ concentration} = \boldsymbol{A\ sample} \ / \ \boldsymbol{A\ standard} \times \mathbf{2.29}\ \boldsymbol{mmol/L}$$

3.9.2 Serum total cholesterol assay

The serum level of TC was determined after enzymatic hydrolysis and oxidation of the sample by the method as described by Richmond (1973) and Roeschlau *et al* (1974). 1000µl of the reagent was added to 10µl each of the sample and standard. This was incubated for 10 minutes at 20-25ºC and the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured against the reagent blank within 30 minutes. The concentration was calculated;

$$\boldsymbol{TC\ concentration} = \boldsymbol{A\ sample} \ / \ \boldsymbol{A\ standard} \times \ \mathbf{5.17}\ \boldsymbol{mmol/L}$$

3.9.3 Serum high density lipoprotein cholesterol assay

The total low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by the addition of phosphotungstic acid in the presence of Magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and then centrifuged for 10 minutes at 4000rpm. The supernatant represents the HDLC fraction in the serum sample. The cholesterol concentration in the HDL fraction, which remains in the supernatant, was also determined.

3.9.4 Serum low density lipoprotein cholesterol determination

The serum concentration of LDLC was calculated in mmol/L using Friedewald's equation (Friedewald *et al*., 1972).

$$\boldsymbol{LDLC = TC - (HDLC + TGL \,/\, 2.2)}$$

3.9.5Antiatherogenic Index (AAI) determination

The AAI was calculated according to the method of Guido and Joseph (1992). It was calculated from the TC and HDLC using the formula below. The value was expressed as a percentage;

$$\boldsymbol{mAAI = HDLC \times 100 \,/\, TC - HDLC}$$

3.10 Effects of the Extract on Kidney Health

Serum creatinine and serum urea levels were determined by the following methods;

3.10.1 Determination of serum creatinine (Jaffe's Reaction)

To about 0.5ml of the serum sample, 1.5ml of distilled water +0.5ml of 10% sodium tungstate+0.5ml of 2/3 normal sulphuric acid was added. The mixture was shaken well and allowed to stand for 15minutes for colour to develop. The optical density of the developed colour was read at 520nm.

3.10.2 Determination of serum urea (Diacetylmonoxime method)

Blood serum was deproteinised using Trichloroacetic acid and the resulting filtrate heated for 20minutes with Diacetylmonoxime reagent in the presence of perchloric acid and allowed to develop a stable yellowish colour. The optical density of the coloured solution was measured at 520nm.

CHAPTER FOUR

4.0 RESULTS

4.1 Acute Toxicity Study

There were no deaths recorded in the first and second phase of acute toxicity test after the extract administration.

4.2 Phytochemical Screening

The phytochemical screening of the methanolic leaf extract of *Tacazzea apiculata* revealed the presence of secondary metabolites such as carbohydrates, tannins, alkaloids, steroids, triterpenes, flavonoids and saponins (Table 4.1).

4.3 Effect of the Methanolic Leaf Extract of *T. apiculata* on Body Weights of Alloxan-Induced Diabetic Rats

The normal control, untreated control and treated diabetic groups showed increase in the mean weekly body weights. These increases were not statistically significant (p □ 0.05). The normal control rats showed less drastic increase when compared with the diabetic untreated or treated groups. The treated diabetic groups showed progressive and steady increase in their mean weekly body weights. (Table 4.2)

Table 4.1; Phytochemical analyses on methanolic leaf extract of *Tacazzea apiculata*

S/no	Test for Secondary Metabolites	Results
1	**CARBOHYDRATES**	
	Molisch s Test	+
2UNSATURATED STEROIDS AND TRITERPENES		
	Libermann-Burchard s Test	+
3	**SAPONNINS**	
	Frothing Test	+
4	**FLAVONOIDS**	
	Sodium Hydroxide Test+	
5	**TANNINS**	
	Ferric Chloride Test	+
6	**ALKALOIDS**	
	Mayer s Test	+
	Dragendoff s Test	+
7	**ANTHRAQUINONES**	
	Borntragers Test	-
	Modified Borntragers Test	-
8	**CARDIAC GLYCOSIDES**	
	Keller-Kiliani Test	-
	Kedde Test	-

Table 4.2: Effects of Methanolic leaf extract of *Tacazzea apiculata* on body weights of Alloxan-induced diabetic rats.

Groups	Weekly body weights (g)				
	Initial	**Week 1**	**Week 2**	**Week 3**	**Week 4**
A	240.80±6.51	244.80±5.54	248.60±6.17	257.60±2.77	257.80±3.31
B	247.00±1.30^{a}	261.60±1.81^{a}	255.60±1.36^{a}	252.60±1.21^{a}	251.00±1.58^{a}
C	225.60±12.05^{a}	224.60±12.93^{b}	216.00±13.20^{b}	211.20±14.40^{b}	217.40±13.75^{bc}
D	236.80±10.54^{a}	227.00±12.26^{b}	229.80±11.93^{ab}	233.60±11.87^{ab}	203.40±8.28^{c}
E	238.80±8.10^{a}	244.20±7.91^{ab}	246.40±10.35^{a}	256.80±9.32^{a}	233.60±12.19^{ab}
F	247.20±4.38^{a}	249.40±5.22^{ab}	255.40±5.03^{a}	253.40±7.62^{a}	251.00±2.92^{a}

Values are mean ± SEM (Standard error of the mean) of the five animals in each group. Means with the same superscripts are not significantly different (P<0.05).

Group A-non-diabetic rats treated with distilled water; Group B-diabetic untreated rats; Group C-diabetic rats treated with 100mg/kg b.w of extract; Group D- diabetic rats treated with 200mg/kg b.w of extract; Group E-diabetic rats treated with 400mg/kg b.w of extract; Group F- diabetic rats treated with Glibenclamide 5mg/kg. b.w.

4.4 Antidiabetic Activity 4.4.1 Hypoglycaemic activity of methanolic leaf extract of *T.apiculata* on bloodglucose level of alloxan-induced diabetic rats.The effect of the oral administration of the extract and Glibenclamide at doses equivalent to 100, 200, 400 mg/kg b.w. of extract and 5mg/kg b.w. of Glibenclamide on the blood glucose level of alloxan induced diabetic rats at the 3^{rd}, 6^{th}, 9^{th}, 12^{th} and 24^{th} hours are shown on Table 4.3.

The oral administration of extract and standard drug Glibenclamide resulted in a time -dependent reduction in the blood glucose of the diabetic rats. The extract at a dose of 100mg/kg b.w. showed a significant hypoglycaemic activity at the 3^{rd} hour after treatment ($p \square 0.05$). This dose of extract-100mg/kg b.w., reduced the blood glucose of the diabetic rats from an initial mean value of 520.00±24.92 mg/dL at 0hr to a mean value of 466.40±32.43 mg/dL at 3hr.The extract at a dose of 200mg/kg b.w. also significantly reduced the blood glucose of the diabetic rats from an initial mean value of 545±15.50 at 0hr to a mean value of 437.80±24.29 at 6hr. While in the untreated diabetic group, the blood glucose increased from the initial mean value of 558.80±7.19mg/dL at the 0hr to a mean value of 559.20±7.34mg/dL at 24hr.The extract at doses 400mg/kg b.w. prevented blood glucose increase, but this was not statistically significant when compared with the untreated diabetic control. The reduction in the blood achieved with treatment with Glibenclamide (5mg/kg b.w.) was not sustained over a 24hr period. These results are presented in Table 4.3.

Table 4.3: Hypoglycaemic Activity of Methanolic Leaf Extract of *T.apiculata* at various times (hours) of Treatment in Alloxan-induced Diabetic Rats.

Groups	Blood glucose level (mg/dl)					
	0th hr	**3rd hr**	**6th hr**	**9th hr**	**12th hr**	**24th hr**
A	132.40±8.39	133.00±7.81	133.60±7.84	132.80±7.30	133.80±7.79	131.20±7.07
B	558.80±7.19^{a}	556.80±7.17^{a}	558.60±7.13^{a}	558.20±6.91^{a}	556.80±6.37^{a}	559.20±7.34^{a}
C	520.00±24.92^{a}	466.40±32.43^{b}	507.60±10.57^{b}	492.80±35.04^{b}	451.60±27.46^{b}	432.00±52.11^{b}
D	545.80±15.05^{a}	504.40±8.05^{ab}	437.80±24.29^{c}	404.80±17.25^{c}	413.60±22.00^{b}	415.80±20.66^{b}
E	541.80±24.51^{a}	501.20±25.12^{ab}	541.20±10.77^{ab}	556.80±12.64^{a}	540.60±12.39^{a}	482.20±12.24^{ab}
F	410.20±1.56^{b}	448.20±5.48^{b}	526.40±8.61^{ab}	526.20±16.89^{ab}	508.60±15.05^{a}	491.40±23.14^{ab}

Values are mean ± SEM (Standard error of the mean) of the five animals in each group. Means with the same superscripts are not significantly different (P<0.05)

Group A-non-diabetic rats treated with distilled water; Group B-diabetic untreated rats; Group C-diabetic rats treated with 100mg/kg b.w of extract; Group D- diabetic rats treated with 200mg/kg b.w of extract; Group E- diabetic rats treated with 400mg/kg b.w of extract; Group F- diabetic rats treated with Glibenclamide 5mg/kg b.w

4.4.2Hypoglycaemic activity of methanolic leaf extract of *T.apiculata* and Glibenclamide at varying times (weeks) of treatment in alloxan- induced diabetic rats.

Administration of the methanolic leaf extract of *Tacazzea apiculata*at different doses 100, 200 and 400mg/kg b.w. and Glibenclamide 5mg/kg b.w. significantly (p 0.05) reduced the blood glucose levels of the diabetic rats when compared with untreated diabetic control at the 1st, 2nd, 3rd and 4th weeks of treatment. The extract at doses of 200 and 400mg/kg b.w. proved to have better blood glucose-lowering effect than the Glibenclamide treated group.

There was however an increase in the blood glucose level of the alloxan-induced untreated diabetic rats when compared with the normal control. The standard drug used-Glibenclamide at 5mg/kg b.w. caused a decline in the blood glucose level through the 4 weeks treatment period, but this reduction was found to be statistically insignificant (p 0.05) when compared to that achieved by the extract, even at the lowest dose100mg/kg b.w. Administration of the three classes of extract doses resulted in a significant decline (p 0.05) hypoglycaemic effects in the diabetic rats at the 2nd, 3rd and 4th week when compared with the untreated diabetic control. Treatment with the extract at the various doses over the four weeks of treatment reduced the blood glucose level of the diabetic ratsto mean values close to or even less than that of the normal control.

The extract at 100mg/kg b.w. dose reduced the blood glucose level of diabetic rats from an initial mean value of 520.00±24.92mg/dLat week 0 to a mean value of 111.60±10.03mg/dL at the 4th week, while the 200mg/kg b.w. dose reduced the blood glucose level of diabetic rats from an initial mean value of 545.80±15.05mg/dLto 115.40±6.62mg/dLand the 400mg/kg b.w. dose reduced the blood glucose level from 541.80±24.51mg/dL to69.40±1.72mg/dL. The final fourth week mean values of 111.60±10.03 and 115.40±6.62 of doses 100 and 200mg/kg b.w. respectively are comparable with the normal control final fourth week mean value of 129.20±3.87 and were more significant than that of the standard drug, Glibenclamide, which reduced the blood glucose from an initial mean value of

410.20±1.56at week 0 to 134.20±4.82at the 4th week. These findings are presented in Table 4.4.

Table 4.4: Hypoglycaemic Activity of Methanolic Leaf Extract of *T. apiculata* at varying times(weeks) of Treatment in Alloxan-induced Diabetic Rats

Groups	Weekly blood glucose level (mg/dl)				
	Initial	**Week 1**	**Week 2**	**Week 3**	**Week 4**
A	132.40±8.39	129.80±4.03	127.20±7.01	123.60±5.28	129.20±3.87
B	558.80±7.19^{a}	560.20±6.95^{a}	558.60±5.90^{a}	560.60±6.71^{a}	565.20±6.83^{a}
C	520.00±24.92^{a}	400.40±48.20^{b}	341.00±48.73^{b}	188.60±49.35^{c}	111.60±10.03^{c}
D	545.80±15.05^{a}	367.40±23.19^{b}	283.40±29.81^{bc}	190.00±12.56^{c}	115.40±6.62^{bc}
E	541.80±24.51^{a}	335.20±12.13^{b}	229.20±9.59^{c}	142.40±13.33^{c}	69.40±1.72^{d}
F	410.20±1.56^{b}	336.40±15.86^{b}	307.60±15.06^{bc}	294.40±43.61^{b}	134.20±4.82^{b}

Values are mean ± SEM (Standard error of the mean) of the five animals in each group. Means with the same superscripts are not significantly different (P< 0.05).

Group A-non-diabetic rats treated with distilled water Group B-diabetic untreated rats; Group C-diabetic rats treated with 100mg/kg b.w of extract; Group D- diabetic rats treated with 200mg/kg b.w of extract; Group E- diabetic rats treated with 400mg/kg b.w of extract; Group F- diabetic rats treated with Glibenclamide 5mg/kg b.w

4.4.3 Effects of methanolic leaf extract of *Tacazzea apiculata* on lipid profile, blood parameters and other biochemical parameters in alloxan-induced diabetic rats.

There was a significant decrease in the plasma total cholesterol level in all diabetic animals treated with doses of the extract or Glibenclamide when compared with the normal control, while there was also a decrease in the diabetic untreated rats. The antiatherogenic index value, HDL: TC (HDL to TC ratio) were significantly (p 0.05) increased in the 100 and 200mg/kg b.w. extract treated groups when compared to the normal and diabetic untreated groups (Table 4.5, Figure 4.1).

A significant decrease ($p<0.05$) was also observed in the serum triglyceride (TRIGS) and low density lipoprotein cholesterol (LDL) levels, while a significant increase ($p<0.05$) in high density lipoprotein cholesterol (HDL) level was observed in all diabetic rats treated with doses of the extract or glibenclamide. The untreated diabetic rats showed a significant increase in TRIGS and LDL levels, and a significant decrease in HDL levels (Table 4.5, Figures 4.2, 4.3, 4.4 and 4.5.

There was no significant change ($p<0.05$) observed in potassium, sodium, chloride and hydrogen carbonate ions levels in all six experimental groups (Table 4.6,Figures 4.6, 4.7, 4.8 and 4.9). There was also no significant change (p 0.05) in the mean serum creatinine levels in all six experimental groups. There was however a significant decrease in the mean serum urea level of the 100mg/kg b.w extract treated group when compared with the normal control and diabetic untreated groups (Table 4.6, Figure 4.10 and 4.11).

There was also no significant change observed in the haematocrit and blood count (White Blood Cells, Red Blood Cells and Platelets Count) in all groups. Table 4.7, Figures 4.12, 4.13, 4.14 and 4.15.

Table 4.5: Effects of Methanolic Leaf Extract of *T. apiculata* on Lipid Profile in Alloxan-induced diabetic rats

GROUPS	TC ($mmoll^{-1}$)	TG ($mmoll^{-1}$)	HDL ($mmoll^{-1}$)	LDL ($mmoll^{-1}$)	AAI
A	5.90 ± 0.81^{a}	1.93 ± 0.03^{ab}	1.55 ± 0.05^{c}	3.46 ± 0.77^{a}	38.04 ± 6.83^{b}
B	4.43 ± 0.24^{a}	2.14 ± 0.08^{a}	1.56 ± 0.02^{c}	2.30 ± 0.44^{ab}	56.32 ± 4.80^{b}
C	4.55 ± 0.42^{a}	1.62 ± 0.06^{c}	2.08 ± 0.12^{a}	1.44 ± 0.39^{b}	94.28 ± 19.36^{a}
D	5.08 ± 0.55^{a}	1.70 ± 0.12^{c}	1.84 ± 0.05^{b}	2.76 ± 0.67^{ab}	62.14 ± 9.34^{ab}
E	4.85 ± 0.35^{a}	1.87 ± 0.10^{ab}	1.54 ± 0.09^{c}	2.23 ± 0.26^{ab}	50.00 ± 9.43^{b}
F	4.82 ± 0.57^{a}	1.91 ± 0.11^{ab}	1.66 ± 0.04^{bc}	2.21 ± 0.67^{ab}	58.95 ± 9.62^{b}

Values are mean ± SEM (Standard error of the mean). Means with the same superscripts are not significantly different ($P<0.05$).

Group A-non-diabetic rats treated with distilled water; Group B-diabetic untreated rats; Group C-diabetic rats treated with 100mg/kg b.w of extract; Group D- diabetic rats treated with 200mg/kg b.w of extract; Group E- diabetic rats treated with 400mg/kg b.w of extract; Group F- diabetic rats treated with Glibenclamide 5mg/kg b.w.

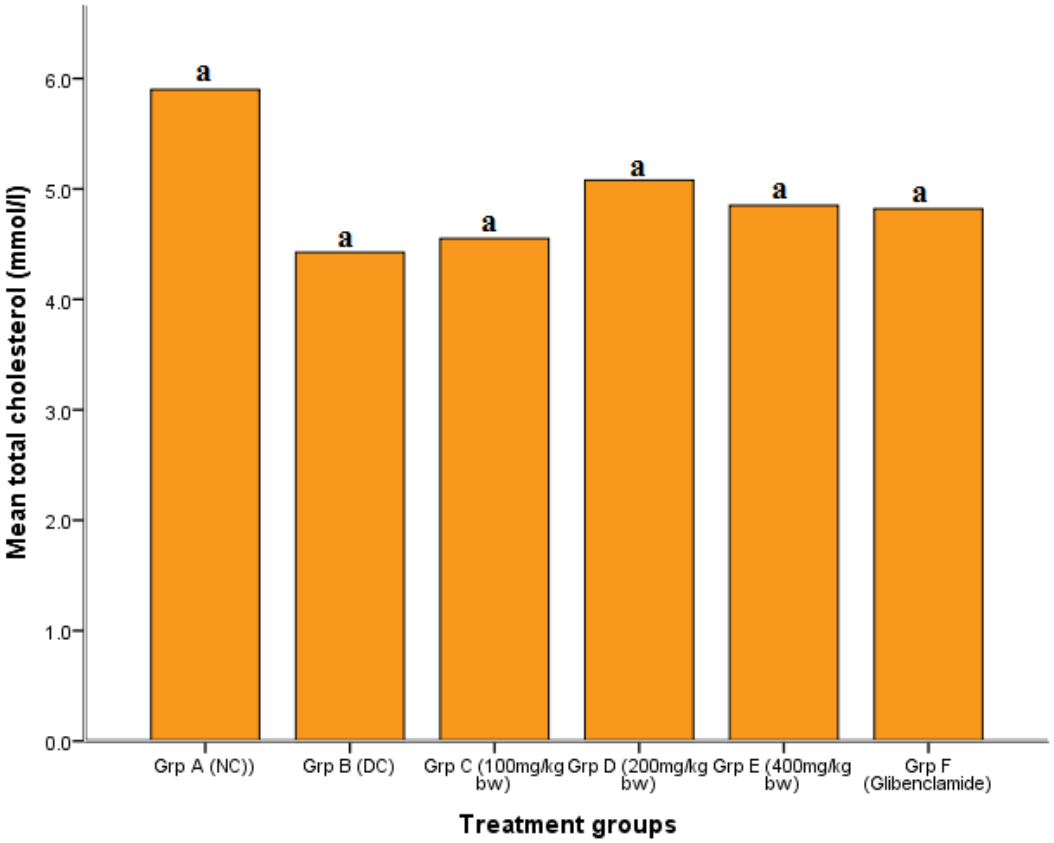


Figure 4.1: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on the Total CholesterolLevel in Alloxan-induced Diabetic Rats

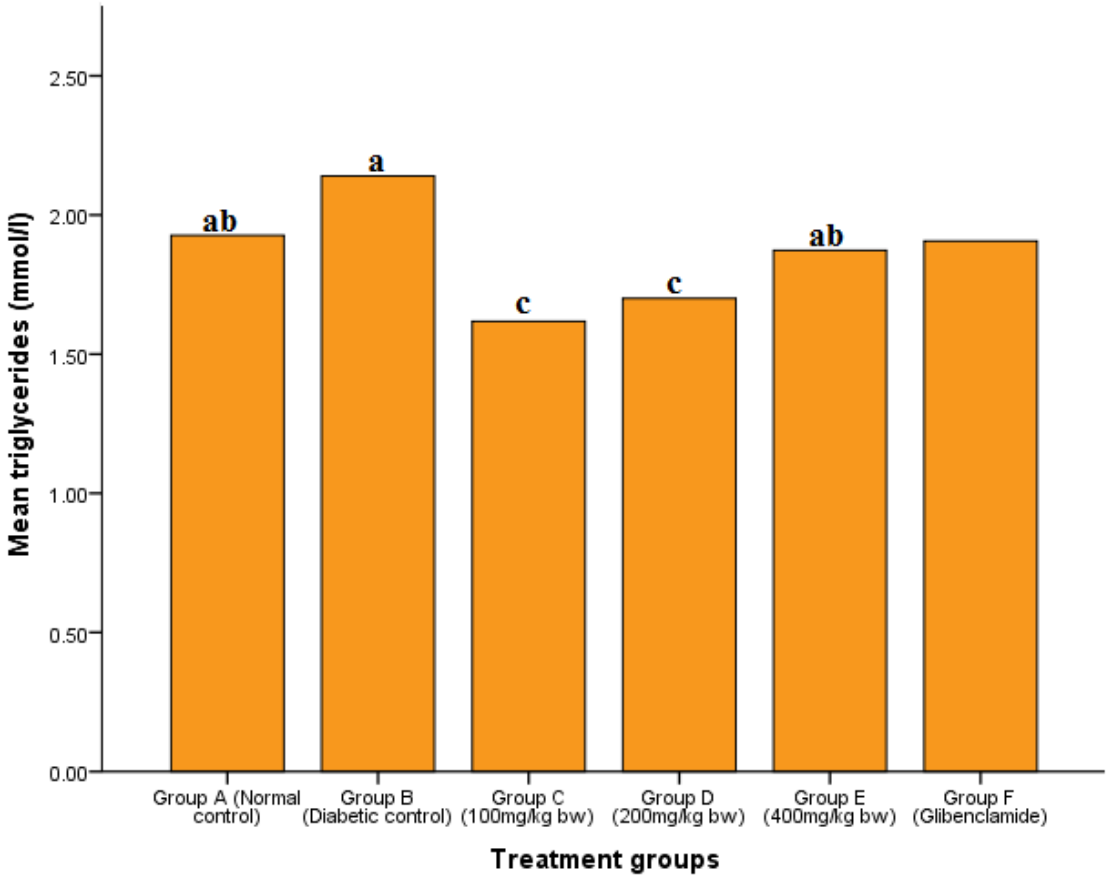


Figure 4.2: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on Triglyceride Level inAlloxan-induced Diabetic Rat.

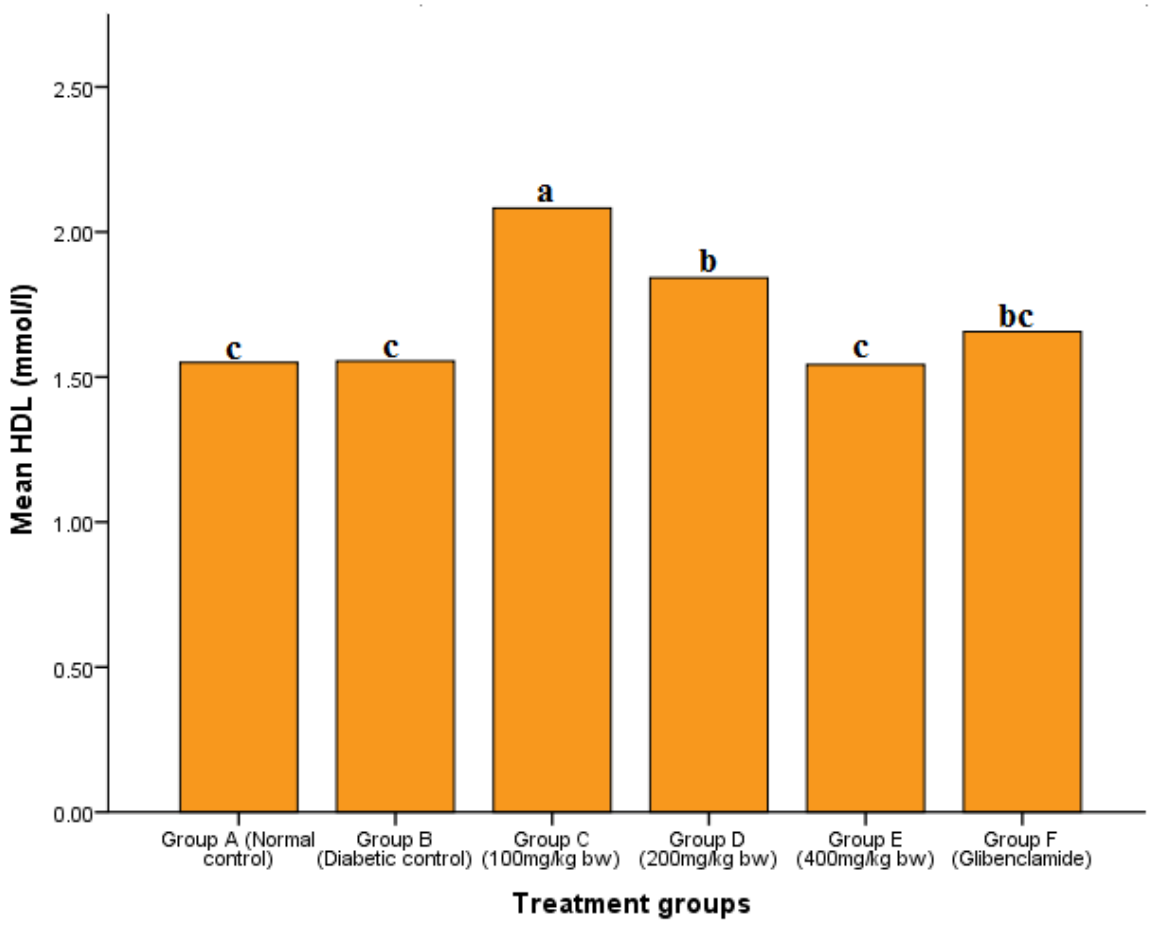


Figure 4.3: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on the High DensityLipoprotein Level in Alloxan-induced Diabetic Rats.

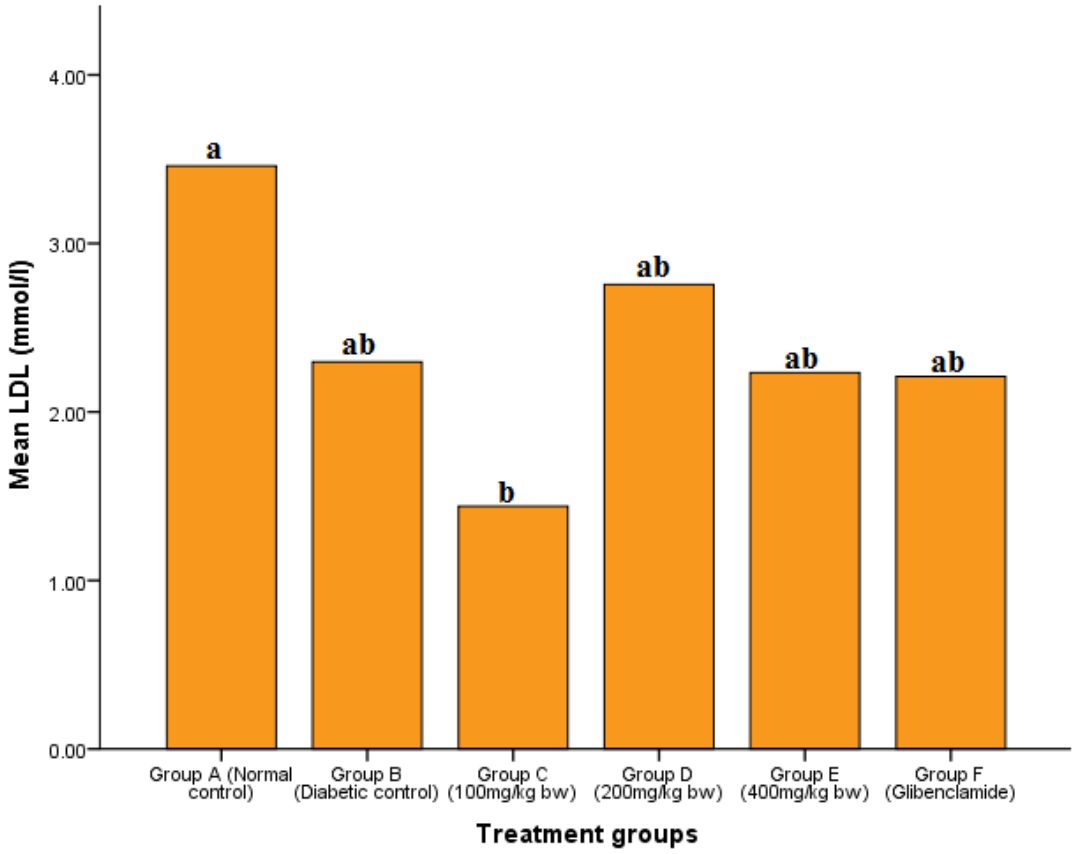


Figure 4.4: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on the Low DensityLipoprotein Level in Alloxan-induced DiabeticRats

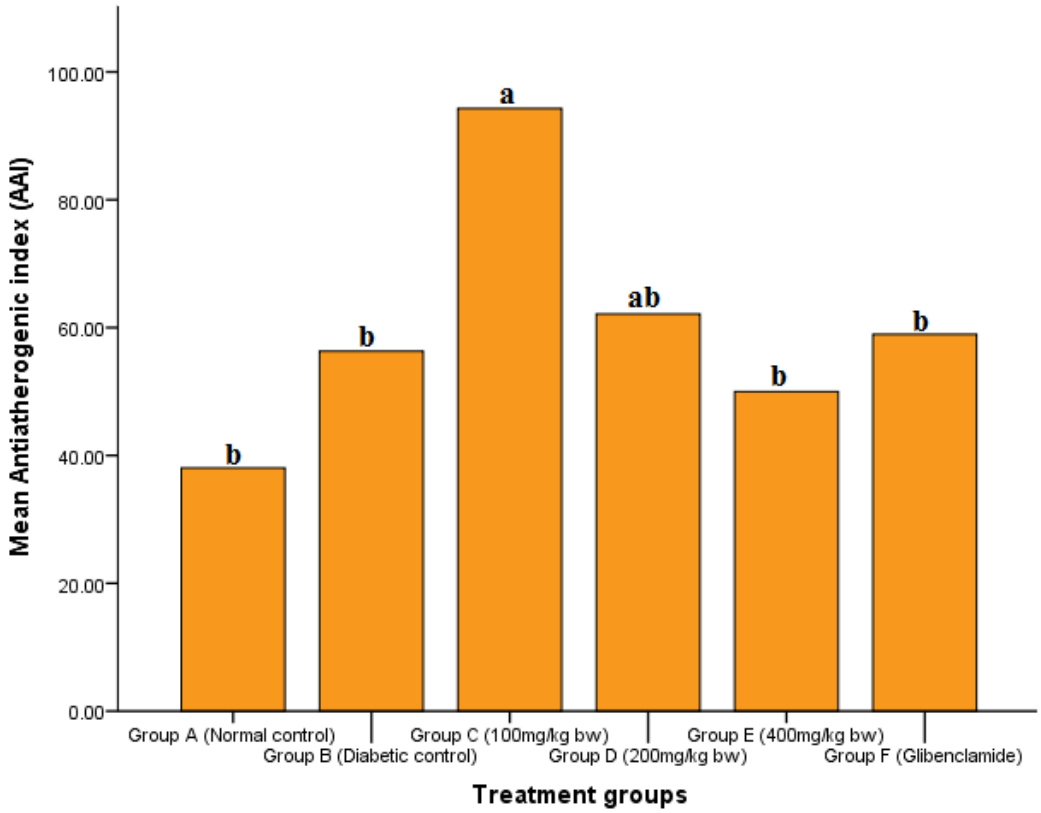


Figure 4.5: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on the Antiatherogenic Index Level in Alloxan-induced Diabetic Rats.

Table 4.6: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on Some Biochemical Parameters in Alloxan-induced Diabetic Rats

GROUPS	Na^{+} ($mmoll^{-1}$)	K^{+} ($mmoll^{-1}$)	Cl^{-} ($mmoll^{-1}$)	HCO^{-3} ($mmoll^{-1}$)	Urea ($mmoll^{-1}$)	Creatinine
A	137.33±1.67^{a}	3.87±0.12^{a}	96.00±2.00^{b}	26.00±0.58^{bc}	4.77±0.49^{bc}	98.67±1.86^{c}
B	138.00±1.08^{a}	3.68±0.17^{a}	96.00±0.71^{b}	26.75±0.48^{b}	4.15±0.25^{c}	100.00±1.08^{bc}
C	141.25±2.06^{a}	3.98±0.09^{a}	100.00±1.58^{ab}	30.25±0.63^{a}	3.05±0.16^{d}	104.25±2.32^{ab}
D	139.00±1.87^{a}	3.62±0.26^{a}	98.80±2.22^{ab}	27.20±0.37^{b}	4.36±0.21^{c}	106.60±2.18^{a}
E	143.75±2.43^{a}	3.68±0.09^{a}	105.50±2.90^{a}	24.50±0.29^{c}	5.28±0.13^{ab}	101.00±0.82^{bc}
F	137.80±1.88^{a}	3.38±0.24^{a}	97.00±2.88^{b}	26.20±0.58^{b}	5.58±0.14^{a}	101.80±0.80^{abc}

Values are mean ± SEM (Standard error of the mean). Means with the same superscripts are not significantly different (P<0.05).

Group A-non-diabetic rats treated with distilled water; Group B-diabetic untreated rats; Group C-diabetic rats treated with 100mg/kg b.w of extract; Group D- diabetic rats treated with 200mg/kg b.w of extract; Group E- diabetic rats treated with 400mg/kg b.w of extract; Group F- diabetic rats treated with Glibenclamide 5mg/kg b.w.

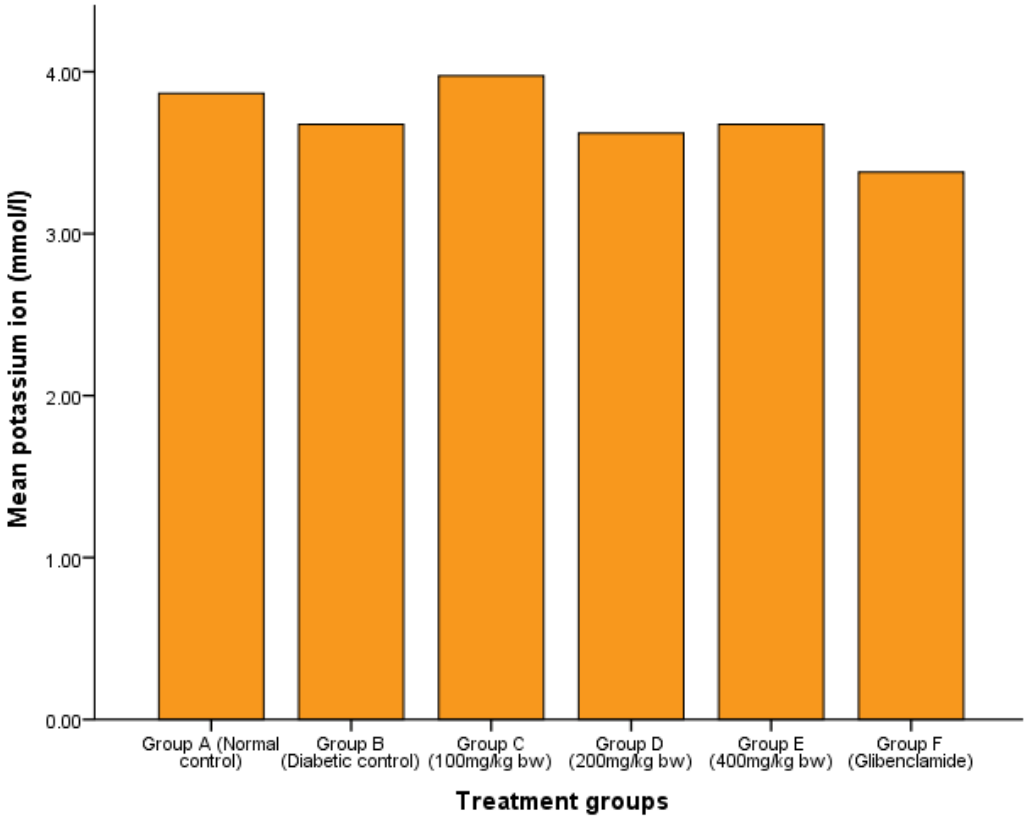


Figure 4.6: Effects of methanolic leaf extract of *Tacazzea apiculata* on the serum potassium level in Alloxan-induced diabetic rats. Note: Vertical bars represent SEM.

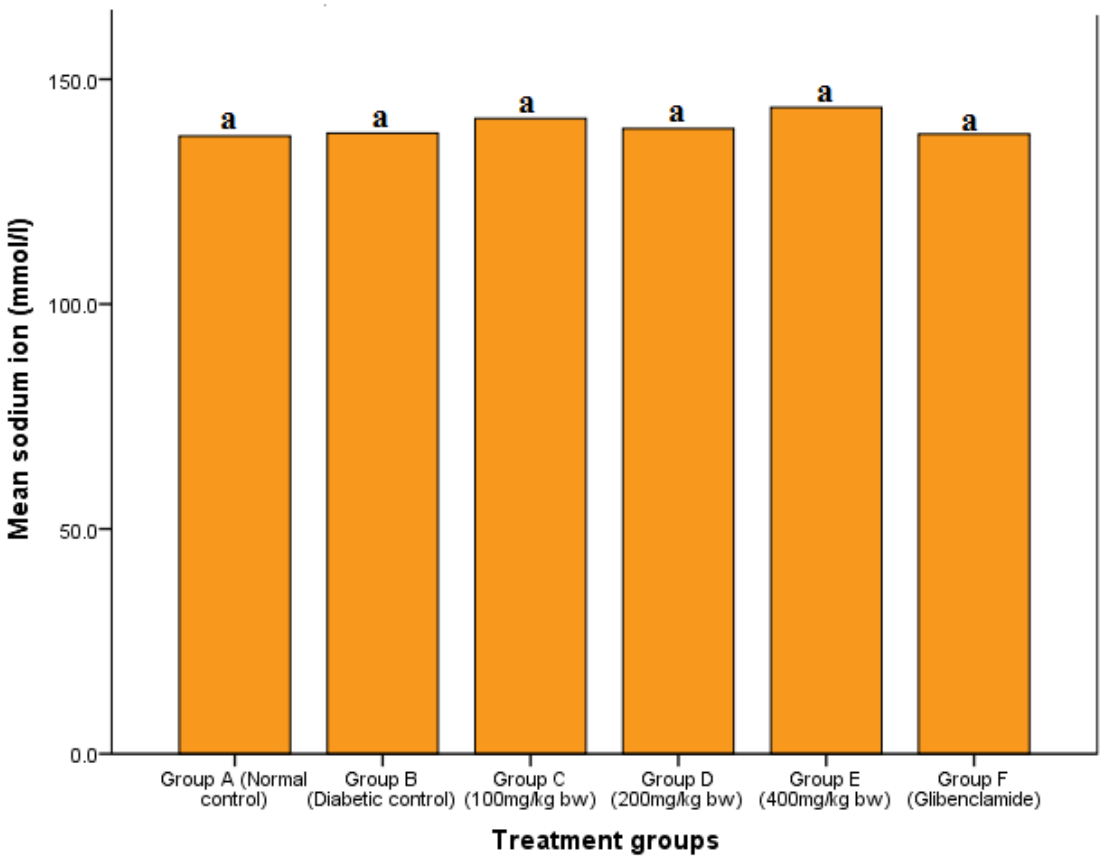


Figure 4.7: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on the Serum Sodium Level in Alloxan-induced Diabetic Rats.

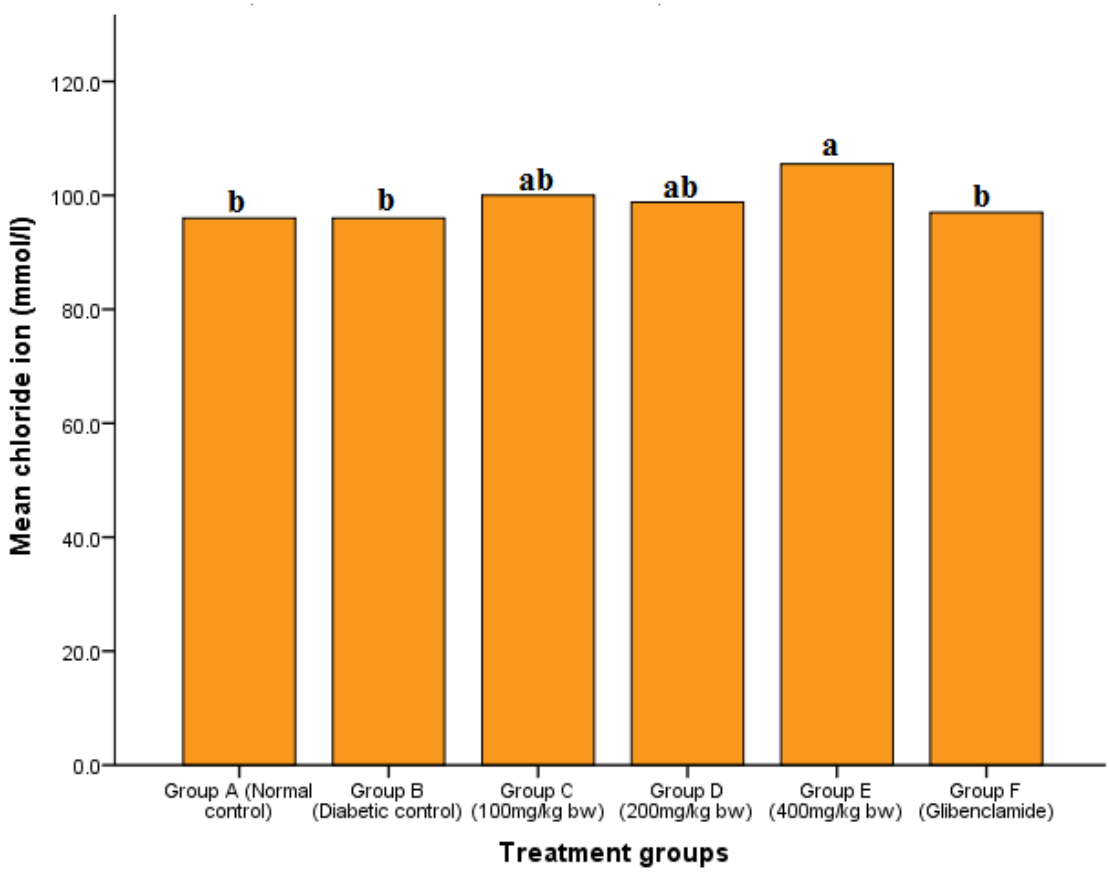


Figure 4.8: Effects of Methanolic Leaf extract of *Tacazzea apiculata* on the Serum Chloride Level in Alloxan-induced Diabetic Rats.

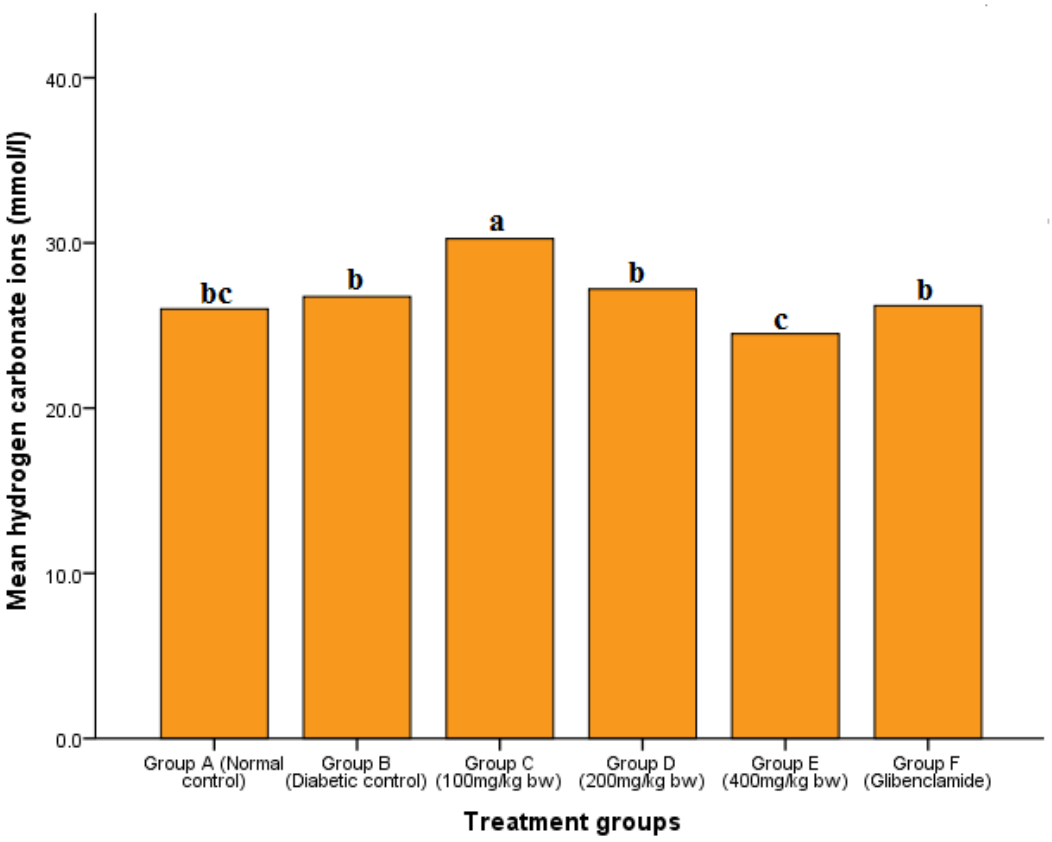


Figure 4.9: Effects of Methanolic leaf extract of *Tacazzea apiculata*on the Serum Hydrogen Carbonate Level in Alloxan-induced Diabetic Rats.

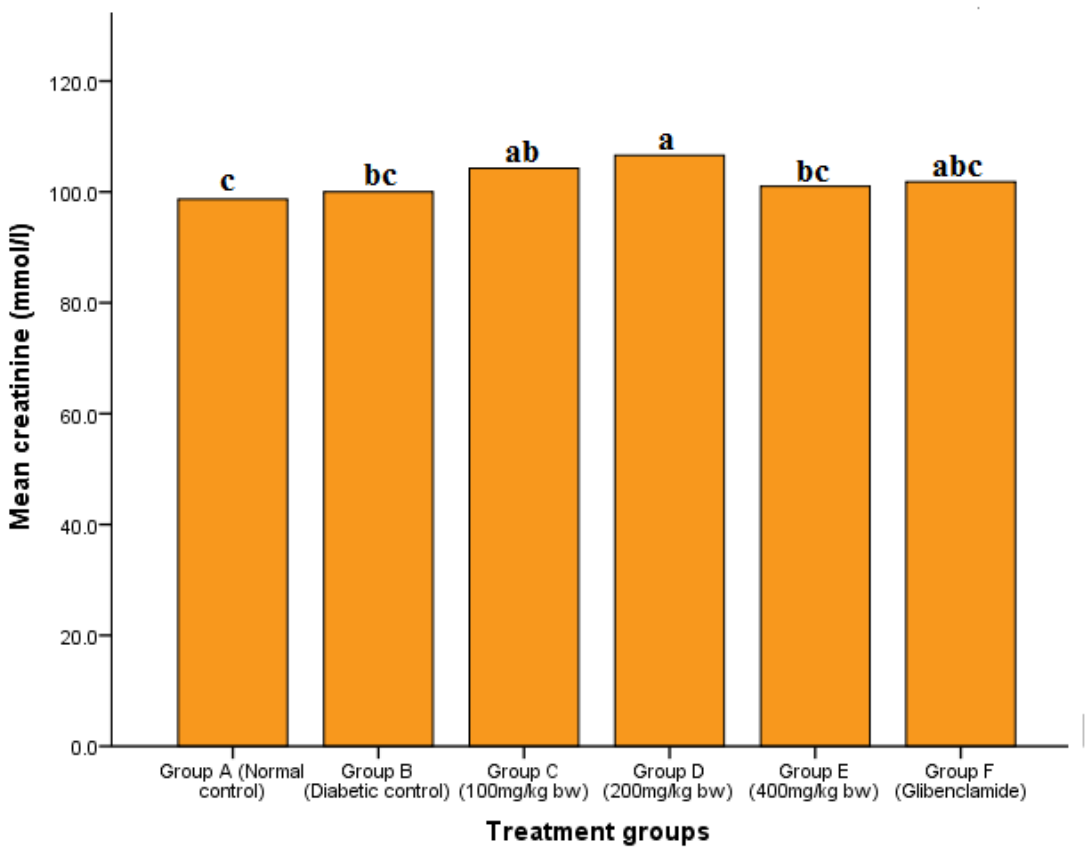


Figure 4.10: Effects of methanolic leaf extract of *Tacazzea apiculata* on the serum creatinine level in Alloxan-induced diabetic rats. Note: Vertical bars represent SEM.

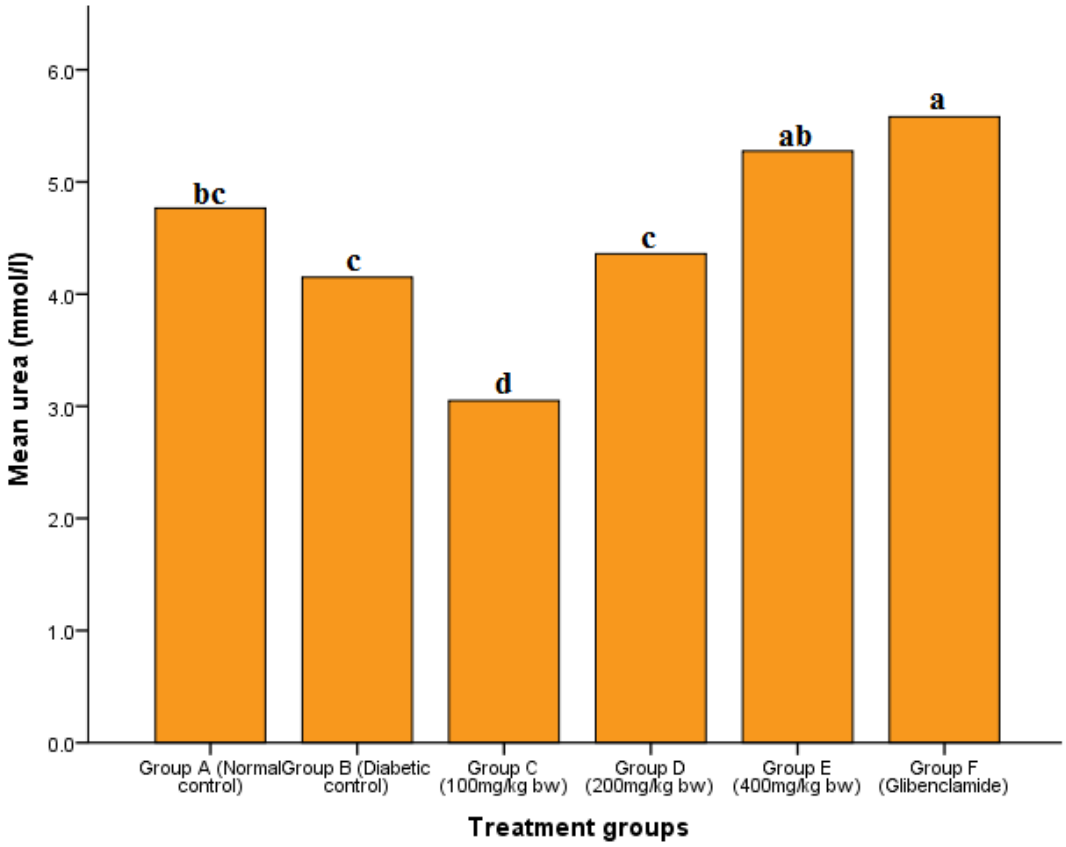


Figure 4.11: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on the Serum Urea Level in Alloxan-induced Diabetic Rats.

Table 4.7: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on Haematocrit and Blood Count in Alloxan-induced Diabetic Rats.

GROUPS	**WBC (µL)**	**RBC (µL)**	**PCV (g/dL)**	**PLT (µL)**
A	20825±5424	7685000±206135[a]	42.4±0.5[a]	700250±77031
B	15333±1753	7296667±156773[a]	40.6±1.9[a]	697000±112434
C	19000±3434	7822500±134001[a]	42.5±0.9[a]	773000±116921
D	15550±2598	5195000±597293[b]	31.6±1.9[b]	537500±96001
E	24000±6150	6600000±913668[ab]	37.1±4.4[ab]	469400±152311
F	14625±3310	7735000±250782[a]	43.0±1.6[a]	593250±12599
***P*-values**	**0.613**	**0.027**	**0.045**	**0.376**

Values are mean ± SEM (Standard error of the mean). Means with the same superscripts are not significantly different at (P<0.05).

Group A-non-diabetic rats treated with distilled water; Group B-diabetic untreated rats; Group C-diabetic rats treated with 100mg/kg b.w of extract; Group D- diabetic rats treated with 200mg/kg b.w of extract; Group E- diabetic rats treated with 400mg/kg b.w of extract; Group F- diabetic rats treated with Glibenclamide 5mg/kg b.w.

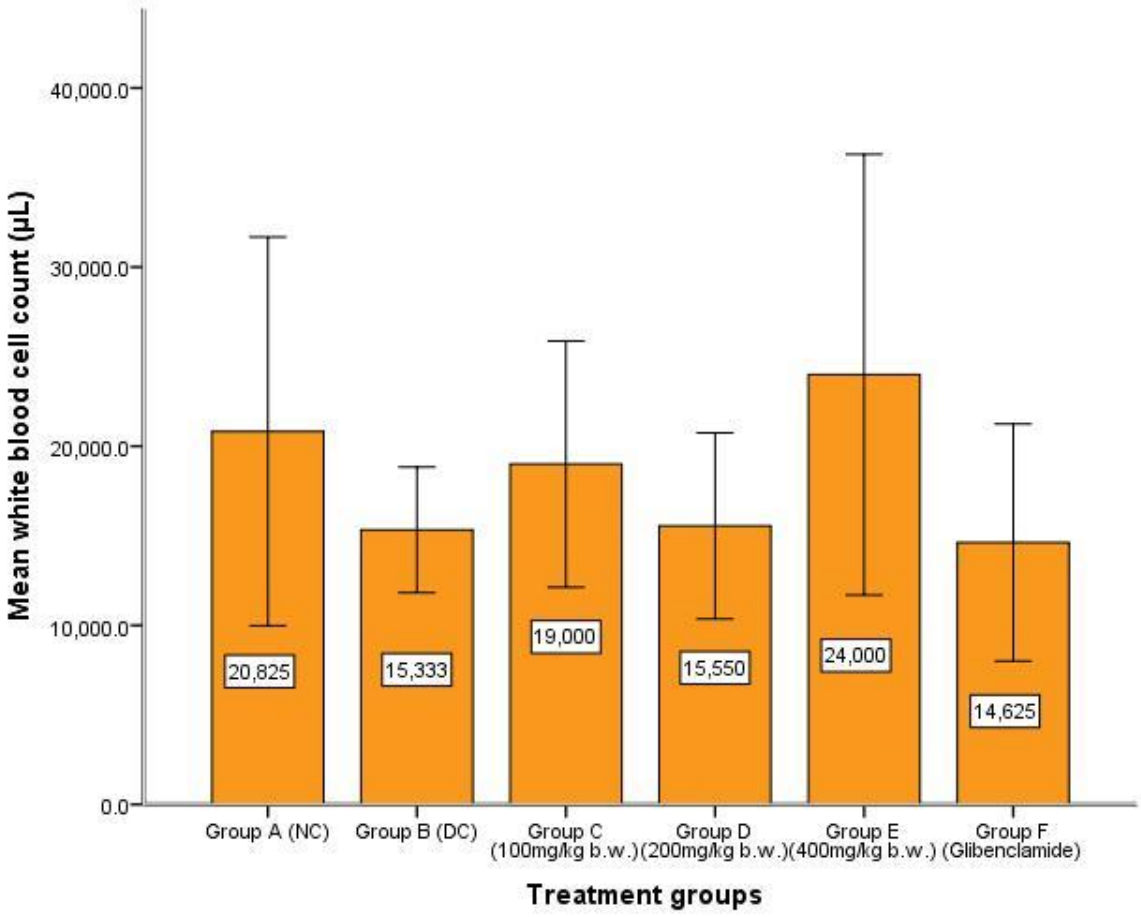


Figure 4.12: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on the Mean White Blood Cell (WBC) Count in Alloxan-induced Diabetic Rats.

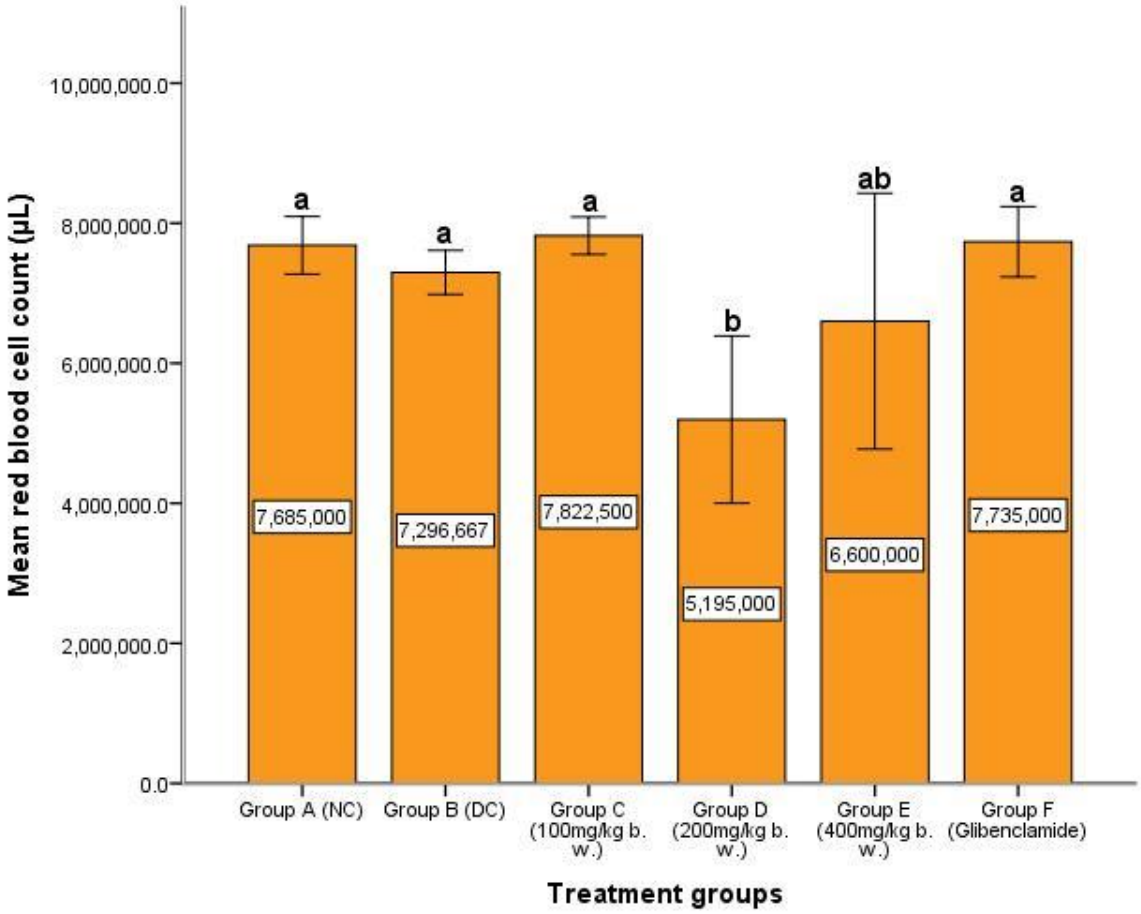


Figure 4.13: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on the Mean Red Blood Cell (RBC) Count in Alloxan-induced Diabetic Rats.

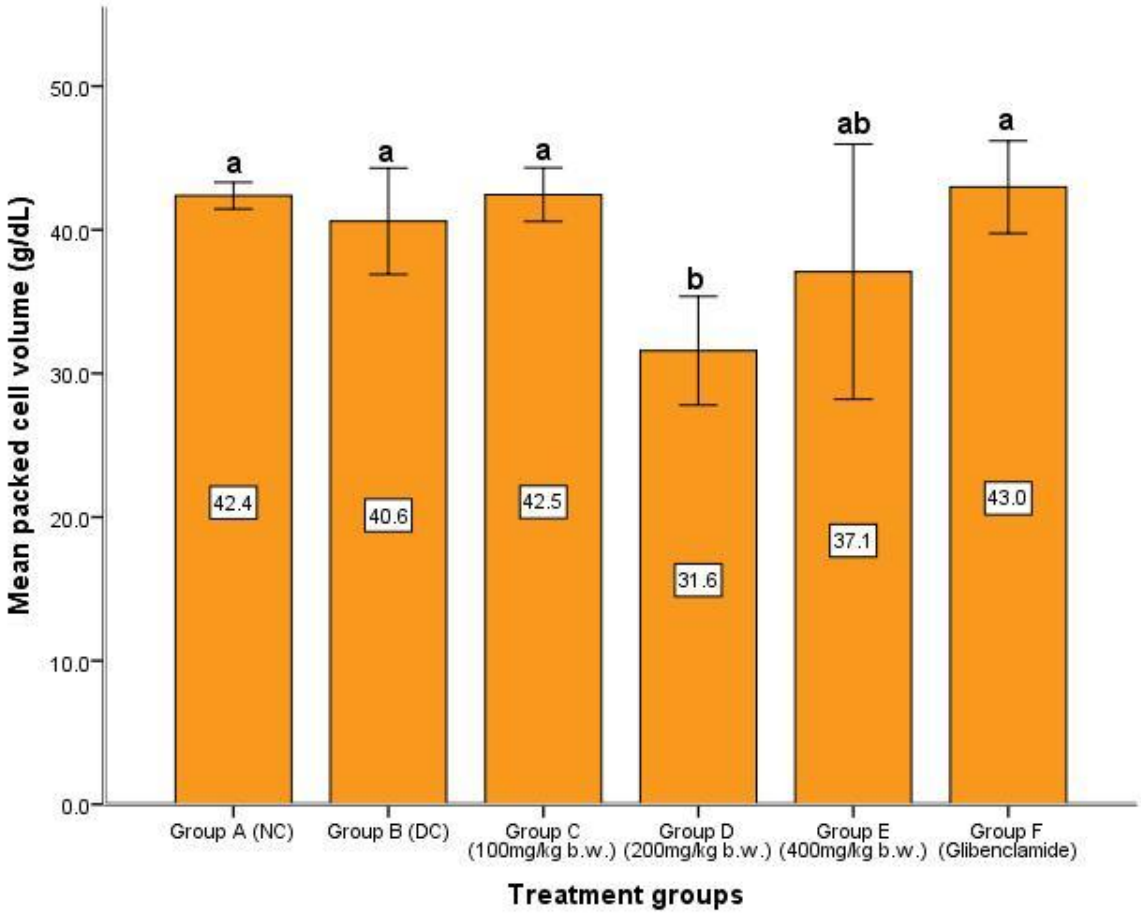


Figure 4.14: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on the Mean Packed Cell Volume (PCV) in Alloxan-induced Diabetic Rats.

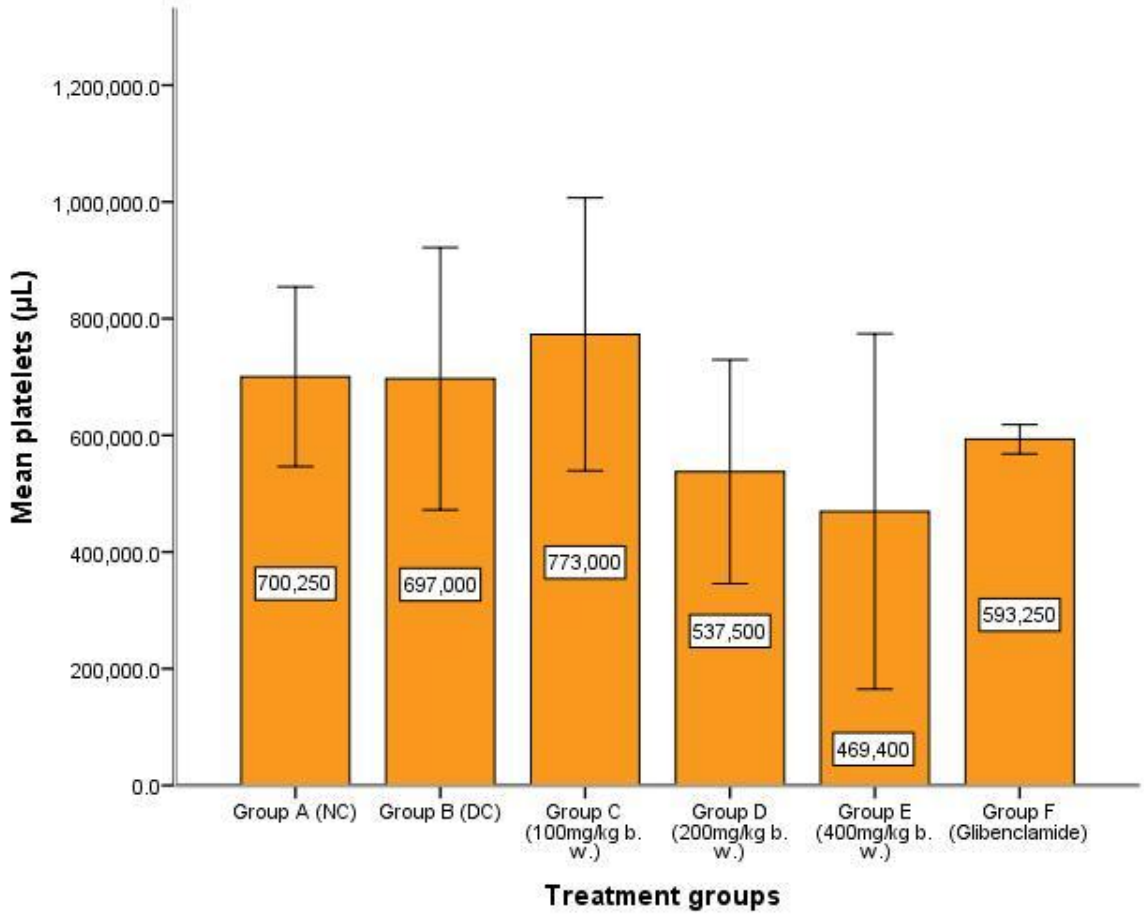


Figure 4.15: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on the Mean Platelet Count in Alloxan-induced Diabetic Rats.

CHAPTER FIVE

5.0 DISCUSSION

The results of this study revealed that oral administration of methanolic leaf extract of *Tacazzea apiculata* had a positive effect on the diabetic state of the experimental animals by reducing hyperglycaemia. Graded variant doses of the extract at 100, 200 and 400mg/kg b.w. caused a statistically significant ($p<0.01$) decline in blood glucose levels in alloxan-induced diabetic rats (Table 4.4). From the result of clinical studies by Knekt *et al*., 2002, it is obvious that the most important factor in the prevention of chronic complications of diabetes mellitus is the reduction of hyperglycaemia. Chronic microvascular complications

include retinopathy, nephropathy, neuropathy, charcot or diabetic foot, accelerated atherosclerosis-related conditions (high blood pressure, myocardial infarction, stroke etc).

Though the exact mechanism of action involved in this observed hyperglycaemic activity is not clear, the extract may have acted by enhancing insulin sensitivity in various organs, especially the muscles, by enhancing glucose uptake and metabolism, thereby inhibiting hepatic gluconeogenesis.

The phytochemical screening of the methanolic leaf extract of *Tacazzea apiculata* indicated the presence of secondary plant metabolites such as; alkaloid, tannins, saponins, steroids, flavonoids and triterpenes. The mechanism by which the extract exerts the hypoglycaemic action may be related to the presence of flavonoids among other secondary metabolites or other bioactive chemical constituents that may be present in the plant extract. These could be the active constituents in a group or as individuals, responsible for the hypoglycaemic activity of the extract(Marles and Farnsort, 1995). Flavonoids have been shown to exert their antioxidant activity by various mechanisms; scavenging or quenching free radicals, inhibiting enzymatic systems responsible for free radicals generation (Blaha *et al*., 2004; Dias *et al*.,2005; Lukacinova *et al*.,2008). Aside being antioxidants, flavonoids have been reported to inhibit sodium-dependent vitamin C transporter 1 (SVCT 1) and glucose transporter Isoform 2 (Glut 2), the intestinal transporters for vitamin C and glucose respectively; leading to a decrease in the intestinal absorption of glucose, hence a decrease in the blood glucose concentration (Song *et al*., 2002). Various researchers have also demonstrated that flavonoids act as reducers of hyperglycaemia by causing the inhibition of renal glucose reabsorption through inhibition of the sodium-glucose symporters located in the proximal renal convoluted tubule in the kidney (Hungo *et al*., 1998; Maghrani *et al*., 2005; Lukacinova *et al*., 2008).

Glibenclamide, the standard drug used, like the plant extract, also produced a significant reduction in the blood glucose level of diabetic rats. It's mechanism of action is exerted mainly by increasing insulin secretion. Glibenclamide only works in diabetics with some remaining active B cells. It binds with the ATP-inhibited K^+ channels in the B cell membranes and inhibit channel activity, depolarizing the B cell membrane and increasing Ca^{2+}influx and hence insulin release (Ganong, 2003). The comparable effect of the plant extract with Glibenclamide in this study may suggest some similarity in their mechanism of action. These findings appear to be in agreement with the earlier suggestion by Jackson and Bressler (1981) that sulfonylureas like Glibenclamide have extra-pancreatic hypoglycaemic mechanism of action, secondary to their causing insulin secretion and the attendant glucose uptake into and utilization by tissues.

Hyperglycaemia and Dyslipidemia as well as Oxidative Stress generally coexist in diabetic subjects. Dyslipidemia includes not only quantitative but also qualitative abnormalities of lipoproteins and plays a significant role in the proatherogenesis of vascular complications in diabetes (Sobasgwi *et al*., 2001; Beckman *et al*., 2002; Rotimi *et al*., 2011). High cholesterol levels and hyperlipidemia are associated consequences of diabetes (Mironova *et al*., 2000; Odetola *et al*., 2006; Iweala and Oludare, 2011). This study showed a decrease in the concentration of total cholesterol, low density lipoprotein cholesterol and triglyceride and an increase in concentration of high density lipoprotein cholesterol in the extract and Glibenclamide-treated rats when compared with the normal controlrats. (Figures 4.1, 4.2, 4.3 and 4.4). Diabetes-induced hyperlipidemia is attributable to excess mobilization of fats from the adipose tissues due to the underutilization of glucose (Krishnakumar *et al*., 2000; Nimenibo-uadia, 2003). The lack of insulin and the elevations of the counter-regulatory hormones lead to activation of enzymes (hormone-sensitive lipase) that stimulate lipolysis and enhanced release of free fatty acids from adipose tissue (Subbiah *et al*., 2006; Rotimi *et*

al., 2011). The fatty acids from adipose tissues are mobilized for energy purposes and excess fatty acids are accumulated in the liver, which are then converted to triglyceride (Suryawanshi *et al*., 2006). The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of unlimited actions of lipolytic hormones on the fat depots (Claudia *et al*., 2006).

Lowering of serum lipids through dietary or drugs therapy seems to be associated with a decrease in the risk of cardiovascular disease in diabetes (Claudia *et al*., 2006). In this study, oral administration of the graded doses of methanolic leaf extract *Tacazzea apiculata* significantly reduced serum levels of total cholesterol, triglyceride, low-density lipoprotein, antiatherogenic index and increased serum levels of high density lipoproteins cholesterol in the alloxan-induced diabetic treated rats. Many dietary factors have been reported to contribute to the ability of herbs to improve dyslipidemia (Nimenibo-uadia 2003; Rotimi *et al*., 2011). Preliminary phytochemical screening of the extract revealed the presence of saponins among other polyphenolic compounds. This may also be responsible for the lipid-lowering effect of *Tacazzea apiculata* on plasma lipid. Saponins are known antinutritional factors, which lower cholesterol, by binding with cholesterol in the intestinal lumen, preventing its absorption, and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase its faecal excretion (James *et al*., 2010; Nimenibo-audia, 2003; Rotimi *et al*., 2011). Increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol (Rotimi *et al*., 2011). Hence, saponins have been reported to have hypocholesterolic effect (James *et al*., 2010). Kumarappan and Mandal (2007) reported that administration of polyphenolic to alloxan-induced diabetic rats reduced hyperlipidemia, and attributed this to a reduction in the activity of hepatic HMG-CoA reductase, which is the first committed enzymatic step in cholesterol synthesis. This lowers elevated LDL cholesterol

levels, resulting in a substantial reduction in coronary events and deaths from coronary heart disease (CHD) that occurs in diabetics (Richard and Pamela, 2009). Thus the observed hypolipidemic effect of *Tacazzea apiculata* can therefore be linked to the synergistic actions of the phytochemicals present in it. It is reported that the derangement of glucose, fats and protein metabolism in diabetes results in the development of hyperlipidemia (Austin and Hokanson, 1994; Brown and Goldstein, 1983). In this study, all doses of the plant extract produced a significant beneficial effect on serum lipid profile in alloxan-induced diabetic rats, the beneficial effects on the lipid profile may be secondary to glycaemia control. The significantly lowered cholesterol may have contributed to theobserved higher levels of high-density lipoprotein cholesterol in the animals. About 30% of blood cholesterol is carried in the form of HDL-C. HDL-C functions to remove cholesterol atheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-C protect against cardiovascular disease (Kwiterovich, 2000; James *et al*., 2010). Therefore, the observed increase in the serum HDL-C level on administration of various doses of the extract in alloxan-induced diabetic rats indicates that the extract have HDL-C boosting effect. More so, the stabilization of serum triglyceride and cholesterol levels in the rats by the plant extract may be attributed to glucose utilization and hence depressed mobilization of fats (Mono *et al*., 2006; Iweala and Oludare, 2011). This result implies that the plant may be helpful in reducing the complications of hyperlipidemia and hypercholesterolemia which coexist quite often in diabetics (Sharma *et al*., 2003). This study also revealed that administration of the extract at various doses significantly lowered the serum LDL-C in alloxan-induced diabetic rats. Studies have shown that chronic insulin deficiency as observed in alloxan-induced diabetes in experimental animals is associated with diminished levels of LDL-C receptors. This results to an increase in LDL particles and consequently increases serum levels of LDL-C (Suryawanshi *et al*., 2006).

The diabetic rats treated with 100,200 and 400mg/kg b.w. extract and 5mg/kg b.w Glibenclamide standard drug treated groups showed steady increase in their mean weekly body weights, while the diabetic untreated group showed less steady increase in their mean weekly body weights (Table 4.2). This could be due to loss of body weightassociated with diabetic patients as a result of increased utilization of fats from the adipose tissue for the generation of energy in the body.

A significant decrease in serum urea was observed in the group treated with 100mg/kg b.w. of extract (Figure 4.11) This suggest that the extract at low dose may be able to prevent altered protein metabolism and/or impaired renal function in diabetes mellitus.

In summary, this study suggests that the induction of diabetes mellitus in rats may be prevented by administration of extracts of *Tacazzea apiculata.* We hypothesized that this effect may be the result of antiradical/chelatory properties of the flavonoids, one of the phytochemicals found in the plant. However, inhibition of renal glucose reabsorption may also be involved in the hypoglycaemic effect of these flavonoids. The lipid-lowering effect of the extract can be associated with saponins, a known antinutritional factor, which lowers cholesterol by binding with cholesterol in the intestinal lumen, thereby preventing its absorption.

CHAPTER SIX
6.0CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Phytochemical screening of *Tacazzea apiculata* showed the presence of tannins, saponins, flavonoids, terpenoids/steroids and alkaloids.Acute toxicity study of the extract suggests its safety up to 5000mg/kg b.w.

It was observed from this study that the methanolic leaf extract of *Tacazzea apiculata* had a significant hypoglycaemic activity in alloxan-induced diabetic rats, in addition to reducing hyperlipidemia and other metabolic aberrations associated with diabetes.

The extract at doses of 100 and 200mg/kg b.w. were more effective in significantly lowering the blood glucose levels of the diabetic rats to near normal, in comparison with the 400mg/kg b.w. dose and the Glibenclamide treatment.

6.2Recommendations

In order to develop this plant (*Tacazzea apiculata*) as an antidiabetic therapeutic agent, which may possibly serve as a cheaper agent instead of currently used expensive, inaccessible and sometimes poorly available oral antidiabetic drugs; the following are recommended;

Further pharmacological and biochemical investigations, including possible clinical trials will clearly elucidate the exact mechanism of action by which *Tacazzea apiculata* exert its effects to develop it as a potential antidiabetic drug.

Estimations of the insulin levels and insulin receptors be carried out on experimental animals, this will give clearer insight into the exact mechanism of action of the antidiabetic effect shown by this plant.

Further and more specific phytochemical characterization of the plant extract should be carried out to identify the specific compound(s) involved in the exhibited antidiabetic and hypolipidemic properties.

Experiments with isolated fractions of the extract on isolated organs should be carried out to further narrow down the specific compounds and the sites of action, involved in the antidiabetic activities.

Chronic toxicity studies of this plant should be done to further ascertain its safety of usage on various body organs.

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APPENDICES

Table 4.1; Phytochemical analyses on methanolic leaf extract of *Tacazzea apiculata*

S/no	Test for Secondary Metabolites	Results
1	**CARBOHYDRATES**	
	Molisch s Test	+
2UNSATURATED STEROIDS AND TRITERPENES		
	Libermann-Burchard s Test	+
3	**SAPONNINS**	
	Frothing Test	+
4	**FLAVONOIDS**	
	Sodium Hydroxide Test	+
5	**TANNINS**	
	Ferric Chloride Test	+
6	**ALKALOIDS**	
	Mayer s Test	+
	Dragendoff s Test	+
7	**ANTHRAQUINONES**	
	Borntragers Test	-
	Modified Borntragers Test	-
8	**CARDIAC GLYCOSIDES**	
	Keller-Kiliani Test	-
	Kedde Test	-

Table 4.2: Effects of Methanolic leaf extract of *Tacazzea apiculata* on body weight of Alloxan-induced diabetic rats

Groups	Weekly body weights (g)				
	Initial	**Week 1**	**Week 2**	**Week 3**	**Week 4**
A	240.80±6.51	244.80±5.54	248.60±6.17	257.60±2.77	257.80±3.31
B	247.00±1.30^{a}	261.60±1.81^{a}	255.60±1.36^{a}	252.60±1.21^{a}	251.00±1.58^{a}
C	225.60±12.05^{a}	224.60±12.93^{b}	216.00±13.20^{b}	211.20±14.40^{b}	217.40±13.75^{bc}
D	236.80±10.54^{a}	227.00±12.26^{b}	229.80±11.93^{ab}	233.60±11.87^{ab}	203.40±8.28^{c}
E	238.80±8.10^{a}	244.20±7.91^{ab}	246.40±10.35^{a}	256.80±9.32^{a}	233.60±12.19^{ab}
F	247.20±4.38^{a}	249.40±5.22^{ab}	255.40±5.03^{a}	253.40±7.62^{a}	251.00±2.92^{a}

Values are mean ± SEM (Standard error of the mean) of the five animals in each group. Means with the same superscripts are not significantly different ($P< 0.05$).

Group A-non-diabetic rats treated with distilled water; Group B-diabetic untreated rats; Group C-diabetic rats treated with 100mg/kg b.w of extract; Group D- diabetic rats treated with 200mg/kg b.w of extract; Group E- diabetic rats treated with 400mg/kg b.w of extract; Group F- diabetic rats treated with Glibenclamide 5mg/kg b.w.

Table 4.3: Hypoglycaemic Activity of Methanolic Leaf Extract of *T.apiculata* at various times After Treatment in Alloxan-induced Diabetic Rats

Groups	Blood glucose level (mg/dl)					
	0th hr	**3rd hr**	**6th hr**	**9th hr**	**12th hr**	**24th hr**
A	132.40±8.39	133.00±7.81	133.60±7.84	132.80±7.30	133.80±7.79	131.20±7.07
B	558.80±7.19^{a}	556.80±7.17^{a}	558.60±7.13^{a}	558.20±6.91^{a}	556.80±6.37^{a}	559.20±7.34^{a}
C	520.00±24.92^{a}	466.40±32.43^{b}	507.60±10.57^{b}	492.80±35.04^{b}	451.60±27.46^{b}	432.00±52.11^{b}
D	545.80±15.05^{a}	504.40±8.05^{ab}	437.80±24.29^{c}	404.80±17.25^{c}	413.60±22.00^{b}	415.80±20.66^{b}
E	541.80±24.51^{a}	501.20±25.12^{ab}	541.20±10.77^{ab}	556.80±12.64^{a}	540.60±12.39^{a}	482.20±12.24^{ab}
F	410.20±1.56^{b}	448.20±5.48^{b}	526.40±8.61^{ab}	526.20±16.89^{ab}	508.60±15.05^{a}	491.40±23.14^{ab}

Values are mean ± SEM (Standard error of the mean) of the five animals in each group. Means with the same superscripts are not significantly different ($P< 0.05$).

Group A-non-diabetic rats treated with distilled water; Group B-diabetic untreated rats; Group C-diabetic rats treated with 100mg/kg b.w of extract; Group D- diabetic rats treated with 200mg/kg b.w of extract; Group E- diabetic rats treated with 400mg/kg b.w of extract; Group F- diabetic rats treated with Glibenclamide 5mg/kg b.w.

Table 4.4: Hypoglycaemic Activity of Methanolic Leaf Extract of *T. apiculata*at various times After Treatment in Alloxan-induced Diabetic Rats

Groups	Weekly blood glucose level (mg/dl)				
	Initial	**Week 1**	**Week 2**	**Week 3**	**Week 4**
A	132.40±8.39	129.80±4.03	127.20±7.01	123.60±5.28	129.20±3.87
B	558.80±7.19^{a}	560.20±6.95^{a}	558.60±5.90^{a}	560.60±6.71^{a}	565.20±6.83^{a}
C	520.00±24.92^{a}	400.40±48.20^{b}	341.00±48.73^{b}	188.60±49.35^{c}	111.60±10.03^{c}
D	545.80±15.05^{a}	367.40±23.19^{b}	283.40±29.81^{bc}	190.00±12.56^{c}	115.40±6.62^{bc}
E	541.80±24.51^{a}	335.20±12.13^{b}	229.20±9.59^{c}	142.40±13.33^{c}	69.40±1.72^{d}
F	410.20±1.56^{b}	336.40±15.86^{b}	307.60±15.06^{bc}	294.40±43.61^{b}	134.20±4.82^{b}

Values are mean ± SEM (Standard error of the mean) of the five animals in each group. Means with the same superscripts are not significantly different (P<0.05).

Group A-non-diabetic rats treated with distilled water; Group B-diabetic untreated rats; Group C-diabetic rats treated with 100mg/kg b.w of extract; Group D- diabetic rats treated with 200mg/kg b.w of extract; Group E- diabetic rats treated with 400mg/kg b.w of extract; Group F- diabetic rats treated with Glibenclamide 5mg/kg b.w.

Table 4.5 Effects of Methanolic Leaf Extract of *T. apiculata* on the Lipid Profile in Alloxan-induced diabetic rats

GROUP	TC ($mmoll^{-1}$)	TG ($mmoll^{-1}$)	HDL ($mmoll^{-1}$)	LDL ($mmoll^{-1}$)	AAI
A	5.90 ± 0.81^{a}	1.93 ± 0.03^{ab}	1.55 ± 0.05^{c}	3.46 ± 0.77^{a}	38.04 ± 6.83^{b}
B	4.43 ± 0.24^{a}	2.14 ± 0.08^{a}	1.56 ± 0.02^{c}	2.30 ± 0.44^{ab}	56.32 ± 4.80^{b}
C	4.55 ± 0.42^{a}	1.62 ± 0.06^{c}	2.08 ± 0.12^{a}	1.44 ± 0.39^{b}	94.28 ± 19.36^{a}
D	5.08 ± 0.55^{a}	1.70 ± 0.12^{c}	1.84 ± 0.05^{b}	2.76 ± 0.67^{ab}	62.14 ± 9.34^{ab}
E	4.85 ± 0.35^{a}	1.87 ± 0.10^{ab}	1.54 ± 0.09^{c}	2.23 ± 0.26^{ab}	50.00 ± 9.43^{b}
F	4.82 ± 0.57^{a}	1.91 ± 0.11^{ab}	1.66 ± 0.04^{bc}	2.21 ± 0.67^{ab}	58.95 ± 9.62^{b}

Values are mean ± SEM (Standard error of the mean). Means with the same superscripts are not significantly different ($P<0.05$).

Group A-non-diabetic rats treated with distilled water; Group B-diabetic untreated rats; Group C-diabetic rats treated with 100mg/kg b.w of extract; Group D- diabetic rats treated with 200mg/kg b.w of extract; Group E- diabetic rats treated with 400mg/kg b.w of extract; Group F- diabetic rats treated with Glibenclamide 5mg/kg b.w.

Table 4.6: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on Some Biochemical Parameters in Alloxan-induced Diabetic Rats

GROUP	Na^{+} ($mmoll^{-1}$)	K^{+} ($mmoll^{-1}$)	Cl^{-} ($mmoll^{-1}$)	HCO^{-3} ($mmoll^{-1}$)	Urea ($mmoll^{-1}$)	Creatinine
A	137.33±1.67^{a}	3.87±0.12^{a}	96.00±2.00^{b}	26.00±0.58^{bc}	4.77±0.49^{bc}	98.67±1.86^{c}
B	138.00±1.08^{a}	3.68±0.17^{a}	96.00±0.71^{b}	26.75±0.48^{b}	4.15±0.25^{c}	100.00±1.08^{bc}
C	141.25±2.06^{a}	3.98±0.09^{a}	100.00±1.58^{ab}	30.25±0.63^{a}	3.05±0.16^{d}	104.25±2.32^{ab}
D	139.00±1.87^{a}	3.62±0.26^{a}	98.80±2.22^{ab}	27.20±0.37^{b}	4.36±0.21^{c}	106.60±2.18^{a}
E	143.75±2.43^{a}	3.68±0.09^{a}	105.50±2.90^{a}	24.50±0.29^{c}	5.28±0.13^{ab}	101.00±0.82^{bc}
F	137.80±1.88^{a}	3.38±0.24^{a}	97.00±2.88^{b}	26.20±0.58^{b}	5.58±0.14^{a}	101.80±0.80^{abc}

Values are mean ± SEM (Standard error of the mean). Means with the same superscripts are not significantly different (P<0.05).

Group A-non-diabetic rats treated with distilled water; Group B-diabetic untreated rats; Group C-diabetic rats treated with 100mg/kg b.w of extract; Group D- diabetic rats treated with 200mg/kg b.w of extract; Group E- diabetic rats treated with 400mg/kg b.w of extract; Group F- diabetic rats treated with Glibenclamide 5mg/kg b.w.

Table 4.7: Effects of Methanolic Leaf Extract of *Tacazzea apiculata* on Haematocrit and Blood Count in Alloxan-induced Diabetic Rats

GROUP	**WBC (µL)**	**RBC (µL)**	**PCV (g/dL)**	**PLT (µL)**
A	20825±5424	7685000±206135[a]	42.4±0.5[a]	700250±77031
B	15333±1753	7296667±156773[a]	40.6±1.9[a]	697000±112434
C	19000±3434	7822500±134001[a]	42.5±0.9[a]	773000±116921
D	15550±2598	5195000±597293[b]	31.6±1.9[b]	537500±96001
E	24000±6150	6600000±913668[ab]	37.1±4.4[ab]	469400±152311
F	14625±3310	7735000±250782[a]	43.0±1.6[a]	593250±12599
***P*-values**	**0.613**	**0.027**	**0.045**	**0.376**

Values are mean ± SEM (Standard error of the mean). Means with the same superscripts are not significantly different ($P < 0.05$).

Group A-non-diabetic rats treated with distilled water; Group B-diabetic untreated rats; Group C-diabetic rats treated with 100mg/kg b.w of extract; Group D- diabetic rats treated with 200mg/kg b.w of extract; Group E-diabetic rats treated with 400mg/kg b.w of extract; Group F- diabetic rats treated with Glibenclamide 5mg/kg b.w.