EVALUATION OF HYPOGLYCEMIC PROPERTIES OF SELECTED HERBS IN ALLOXAN INDUCED DIABETIC RATS

By

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(Ph.D/SSSE/1999/025)

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DECLARATION

I hereby declare that this research thesis is my original work and to the best of my knowledge has not been presented in any form for the award of degree or any other certificate in any other institution.

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04/05/2010

Date

CERTIFICATION

This thesis titled: "*Evaluation of Hypoglycemic Properties of Selected Herbs in Alloxan induced Diabetic Rats*" by SAIDU, Abubakar Ndaman (Ph.D/SSSE/1999/025) meets the regulation governing the award of the degree of Doctor of Philosophy (Ph.D) in Biochemistry of Federal University of Technology, Minna, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

Dedicated to my late parents Alhaji Saidu Bida and Aminat.

ABSTRACT

Plant parts from six different medicinal plants namely: *Blighia sapida*, *Anacardium occidentale*, *Moringa oleifera*, *Zyzzipus spinachristi*, *Artemisia herba alba* and *Terminalia glauscecens* were analyzed for hypoglycemic properties. The aqueous extract of *A. occidentale* stem bark had the highest crude extract (80%) while that of *Zyzzipus spinachristi* leaves had the lowest crude extract (20%).The aqueous extract of *A.occidentale* leaf had the lowest safe dose (300mg/kg.bw) and lethal dose (450mg/kg.bw) while that of *Zyzzipus spinachristi* leaves had the highest safe dose (900mg/kg.bw) and lethal dose (1,250mg/kg.bw). Phytochemicals such as saponins, tannins, alkaloids, terpenes, flavonoids, glycosides, resins and anthraquinones were detected in some plants analyzed. The aqueous crude extract of *A. occidentale* (300mg/kg.bw) had the highest hypoglycemic activity (74.2%) while that of *A. herba alba* (525mg/kg.bw) had the lowest activity (43.4%) in alloxan induced diabetic rats compared to the other plants. In the stepwise fractionation, the ethanolic fraction of *A. occidentale* leaf extract at 200mg/kg.bw had the highest percentage glucose reduction (46.2%) in diabetic rats compared to other fractions at same dose. However, the fractions from the stem bark extract of *A.occidentale* had low activities in diabetic rats. The fractions also had inhibitory effects on the bacterial species tested namely: *Salmonella typhi,Staphylococcus aureus* and *Kliebsella pneumonae*. On further purification with column chromatography, the ethylacetate fraction of the *A.occidentale* leaf extract at varying doses had the best hypoglycemic activity (59.6% and 54.1%) in diabetic rats compared with other fractions and the standard drug-metformin. The sub chronic toxicity tests revealed a significant ($P<0.05$) decrease in the ALT, AST and protein levels but significant ($P<0.05$) increase in the creatinine and urea levels. The histopathology investigation showed lesions in the liver and kidney tissues at dose ≥ 2000mg/kg bw of ethanolic extract of *A.occidentale* leaf. The spectral analyses of the most active fraction (ethylacetate) revealed the presence of esters namely: 1, 2-Benzenedicarboxylic, mono (2-ethylhexyl) ester and Bis (2-ethylhexyl) phthalate. The results indicate that all the plants had significant hypoglycemic and antibacterial potentials which may be exploited for further research that could lead to drug development.

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CHAPTER ONE

INTRODUCTION

1.0 INTRODUCTION

The scope of medical practice changes as diseases, therapies and prognoses are constantly shifting. One major change in the field of health care is in the treatment of diabetes which is consuming an increasingly large portion of National Health Care expenditures and effort especially in developed countries. It is alarming to note that approximately one – third of cases of diabetes are currently undiagnosed (Michael, 2006). Essentially, diabetes is a non – communicable disease of endocrine origin and metabolic target that affects both adults and children of all races. It is commonly associated with markedly increased morbidity and mortality rates which results in significant financial burden e.g. 92 billion dollars per year in United States (Forster, 1994). Seemingly everywhere, the prevalence of diabetes has increased steadily over the past several decades. In Nigeria, the literature relating to prevalence of the disease is scarce. However, it was reported that over 5 million people are affected as at 2008 (Daily Trust, September 4th,2009).

In Port-Harcourt for example, it was found to be as high as 23.4% among the high socio – economic group and 16% among the low socio – economic group. (Nwafor and Owhoji, 2001). The Centres for Disease Control and Prevention estimates that 30 million people worldwide had diabetes in 1985. A decade later, the global burden of diabetes was estimated to be 135 million. Although, changes in the definition of diabetes may have affected the number (Acog, 1986). The latest World Health Organization (WHO) estimate for the number of people with diabetes, worldwide in 2000 is about 177 million. This is likely to increase to at least 370 million by 2030 (WHO, 2002). Two major concerns are that, much of this increase

will occur in developing countries, due to population growth, ageing, unhealthy diets, obesity, sedentary life-styles and that there will be growing incidence of the disorder. By 2030, while most people with diabetes in developed countries will be aged 65 years or more, in developing countries, the majority will be in the 45 – 65 years age (WHO, 2002). In the United States alone, there are 20.8 million children and adults with diabetes and from the number, 14.6 million have been diagnosed while 6.2 million are unaware that they have the disease. Based on death certificate data, diabetes contributed to 224,092 deaths in 2002 and studies indicate that diabetes is generally under-reported, particularly in cases of older people with multiple chronic conditions such as heart disease and hypertension. As a result, the toll of diabetes is believed to be much higher than officially reported. The total estimated cost of treatment of diabetes in 2007 is 174 billion dollars (Janghorbani *et al*, 2007). It is the fourth leading deadly disease worldwide.

Traditional medicinal remedies for several diseases abound in most countries and it is estimated that some species of higher plants are used medicinally throughout the world. From the early days of crude tea to the sophisticated drugs made from extraction today, plants have long been used by humans as a medicinal source. Many of these preparations have been used extensively and knowledge about them has been accrued by several generations of practitioners from experience, trial and error. Although formal toxicology studies are limited, most extensively used local remedies are unlikely to be severe toxins and are worthy of further evaluation for novel chemotherapeutic compounds. It has been estimated that almost two thirds of the earth is 7% about 6.1 million people rely on these preparations because of the limited availability or affordability of pharmaceutical

medicines (Taylor, 2003). In industrialized countries where scientifically formulated drugs are readily available, there is increasing confidence in the growing use of non-prescriptive drugs from plant sources. In the United States, it was estimated that 5 billion dollars was spent on such preparations in 1997 (Swerdlar, 2000). The acceptability and consumption of such natural health products presently enjoys a commercial boom in African countries especially Nigeria. Despite the promise of plants as cures for many diseases today, fewer than 5% of all tropical plants have been investigated for their medicinal value (Robert, 2003).

Herbal preparations and orthodox drugs are inherently linked and the association dates back to centuries. Dropsy is a condition in which the efficient working of the heart leads to oedema and was effectively treated for centuries in England with a decoction of a mixture of 20 plants. William Withering discovered in 1785 that the only active ingredient was the leaves of foxglove (*Digitals purpurea* L.) (Taylor, 1965; Le Strange, 1977). Since then, this plant and the related species *D. lanata* L. have been thoroughly investigated and their pharmacologically active constituents are now known to be steroidal glycosides having cardiotonic activity. Two of such active constituents from D. purpurea are digoxin and digitoxin both of which are now registered drugs in the British and other pharmacopea.

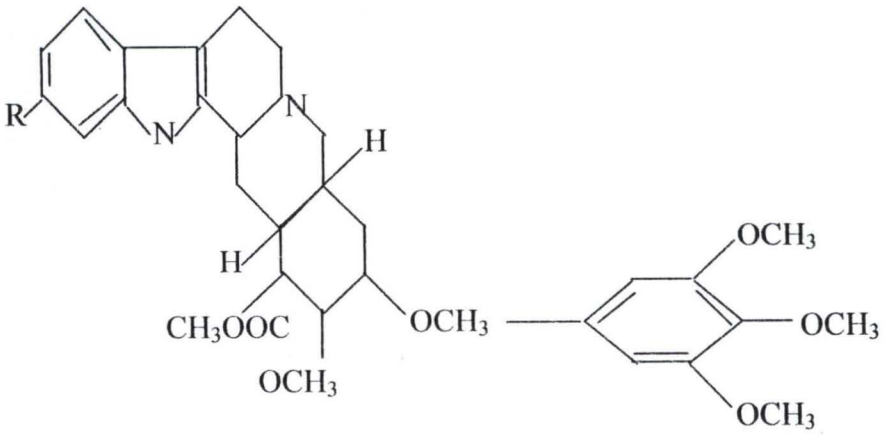
Digitalis itself is still a worldwide remedy for congestive heart failure. In fact, the use of prepared *digitalis* is preferable because the pure compounds (digoxin and digitoxin) are much more dangerous than the crude leaf. The South American, Indians used to poison their arrows with a dark brown or black paste called curare, made from a decoction of mixture of plants belonging to the *Loganiaceaea* and *Menispermaceae* families such as strychnos, toxifera, *S. castelnaei, S. jobertianau*

e.t.c. The Menispermaceous plants used were mainly chondrodendron species especially *C. tomentosum* and possibly also *C. microphylla L., C. platyphylla* and also *Anomospermum grandiflora* (Trease and Evans, 1978).

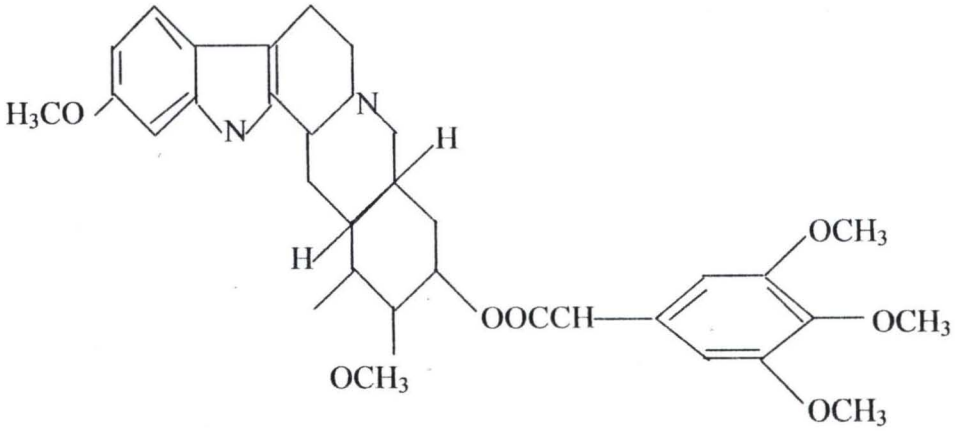
Scientists observed that these arrow poisons paralysed the animals but did not necessarily kill it. The poisons were found to be rich in alkaloids, the most important being D – tubocurarine. This alkaloid has muscle – relaxant properties and is still registered in many pharmacopea as the salt tubocurarine chloride. The drug, in the form of an injection is used as a muscle relaxant in surgery (as an adjunct to anaesthsia) and in certain neurological conditions. The semi-synthetic derivative, dimethyltubocurarine iodide is also available. The Tubocurarine is still extracted from plants because large scale chemical synthesis of the alkaloid is not profitable (Sofowora, 1982).

Over a very long period, the root of *Rauwolfia serpentine* has been used in Indian traditional medicine to treat the mentally ill (Taylor, 1965). The drug was however, more popular as an antidote for snake bite and the sting of scorpions. These latter uses were probably derived from the "doctrine of signatures" as the root has the shape of a snake and would therefore be assumed by the local people to cure snake bite. Unfortunately, this is one example where the doctrine of signatures failed, Rauwolfia's active principles having no antidote properties against snake bite or scorpion sting. The related species *R. vomitoria* (African Rauwolfia) has been used to sedate mentally disturbed patients by traditional medical practitioners in Nigeria and in many African countries for centuries (Dalziel, 1956; Watt and Breyer – Bradwijk, 1962; Kokwaro, 1976). Over 100 alkaloids have been isolated from the

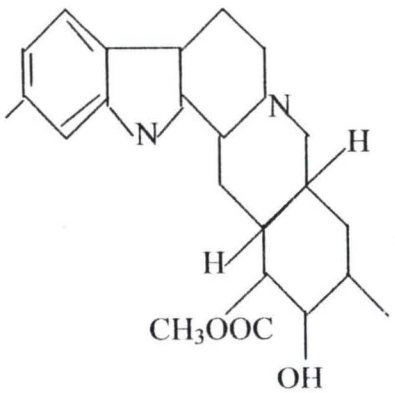
roots of Ranwoltia) species including the therapentically important reserpine rescinamine, deserpidine and yohimbine. Reserpines, together with some of the



Reserprine



Rescinnamine



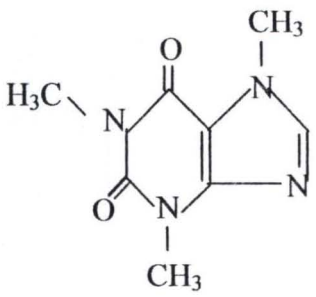
Yohimbine

other Rauwolfia alkaloids have been shown to be responsible for the sedative properties of the roots. These alkaloids also exhibit antihypertensive properties and used in modern medical practices to treat certain cases of anxiety. Large scale production of this alkaloid form the root of Rauwolfia is much deeper than the synthetic process and much preferred.

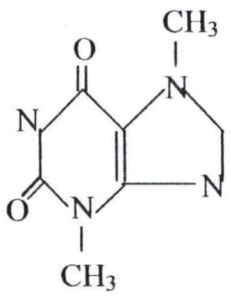
The Madagascar periwinkle, Catharantus roseus G. has long been used as an infusion in traditional medicine in Madagascar, South Africa, Philipines, Jamaica and India for treating diabetes. It is included in Madagascar pharmacopea of crude drugs and is a major source of income for that country and its being cultivated on a large scale for export.(Ratsimamanga, 1980). The anti-lenkemik properties of the plant were discovered by a group of Canadian scientists from 1966 to 1960 while trying to prove that the plant was anti-diabetic (Sim, 1971b; Taylor and Farnsworth, 1973). Further work by Eli Lilly Company in USA led to the isolation of series of dimeric alkaloids together with extracts of the plant possess antilenkaemic properties, the pure alkaloids being preferred for therapeutic purposes. In all, over a 100 alkaloids have now been isolated from C. roseus (Taylor and Faraswoth, 1973; Trease and Evans, 1978; Haidet, 2003). Vinblastine sulphate and Vincristine sulphate are both available as injections and serve nowadays as drugs of choice for leukemia chemotherapy.

The consumption of beverages dates back to ancient times (Taylor, 1965). The most common of them being made from aqueous extracts of coffee, tea and cocoa. For coffee, the seeds of coffee *Arabica L.* and related species are known to contain caffeine. For tea, the leaves of the *Sinensis L.* contain caffeine and theophylline while cocoa, the seeds of *Theobroma Cacao L.* contains caffeine and

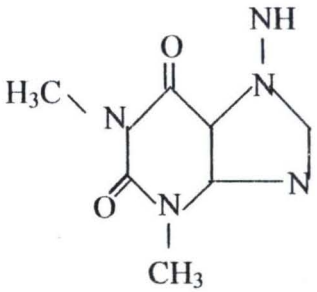
theobromine. Caffeine stimulates the cardiac muscles, acts on the kidney to induce diuresis and produced coronary dilation as well as relaxation of skeletal muscles. Theobromine, a central nervous system and respiratory stimulant also relaxes smooth muscles, while the actions of theophylline are intermediate between those of caffeine and theobromine. All these alkaloids are methylated xanthines and are presently obtained by total chemical synthesis (Famsworth and Moris, 1976).



Caffeine



Theobromine



Theophylline

The discovery of the medicinal qualities of opium was lost in antiquity but opium as a sleep inducing drug was well known to the ancient people being referred to as the joy plant by the Sumerians as early as 4000 BC (Taylor, 1965). Serturnor first isolated the alkaloid morphine from opium in 1806 (Taylor, 1965; Le Strange, 1977; Trease and Evans, 1978). About 25 alkaloids have been isolated from the opium poppy (*Papaver somniferu, L*) but the main alkaloids of therapeutic importance are: morphine, codeine, thebaine, noscapine, narceine and papaverine. Many derivatives of the opium alkaloid have been synthesized and are used in modern medicine, but morphine and cordeine continue to be important sleep inducers and pain killers respectively. Important synthetic analogues include;

pethidine, heroine and narlophine. Morphine and heroin are well know as drugs of addition.

Cocoa leaf, *Erythroxylon coca L.* was chewed by South American Indians in the past to prevent hunger and increase endurance whilst the labourers worked in the field. Godeke in 1855 and later Niemann in 1859 discovered the active principles on the alkaloid cocaine, Niemann named this alkaloid cocaine (Taylor, 1965, he strange, 1977, Trease and Evans, 1978). Because of its toxic nature, the leaf is seldom used but its alkaloid cocaine is employed as a local anaesthetic in the form of its hydrochloride, benzoate or borate. In the form of an elixir or eye drops, cocaine hydrochloride was at one time registered in US and British pharmacopea (Softwora, 1982).

Recently, it was observed that many HIV/AIDS patients who survived the infection either used herbal therapy or complemented their antiviral therapy with plant based nutracenticals (Iwu *et al.*, 2003). It is significant to note that most of these herbal medicinal products were derived from recipes on their activities, inhibitors of HIV Reverse Transcriptase. These vary in their chemical structures including alkaloid, flavonoids, coumarines, Naphthoquinones, anthraquinones, polysaccharides and terpences (Jung *et al.*, 2000).

The most important include the naphtoquinones – Michellamines A to C isolated from the tropical plant *Ancistrocladus korupensis* obtained from Cameroon. Calanolide A, isolated from *Calophyllum lanigerum,* betalinic acid from Syzigium claviforum, the dibenzylbutadience lignans from *Anogeissus accuminata*, the sulphated polysaccharides found in many seaweeds and putanijivain A from the Egyptian plant *Embelica officinalis* e.t.c. (Matthee *et al.*, 1999).

The mode of action of several plant derived agents have not been determined but some have been shown to exhibit significant activity against retrovirus and also act as immuno – modulatory agent such compounds studied under clinical setting include castospermine, glycyrrhizin, paparerine, trichosanthin, aceramannan, albrein (from Aloe barbadensis) and N-butyl-1-dioxynojirimicin (Vlietina et al, 1998). In a study with kolaviron, a mixture of C – 3 and C – 8 linked biflawonones GB1, GB2, kolaflavonone and the benzophenones, kolanone found in the seeds of the West Africa tree Garcina Kola, a dose dependent activity against certain viruses were demonstrated. This plant used in Africa folk medicine is an ingredient in commercial herbal formulations as an "immune tonic" (Iwu *et al.*, 1987, 1990).

The next group of anti-viral drugs derived from plants includes antianaemic agents, products for opportunistic infections and biostimulators (Volberding, 2000). Remarkable results were reported with well known phytonutrients like *Moringa oleifera* and *Sorghum bicolar* (Iwu *et al.*, 2003).Natural compounds with antidiabetic activity include complex carbohydrates,alkaloids,glycopeptides,terpenoids,peptides and amines,steroids,flavonoids,lipids,coumarins,sulfur compounds,inorganic ions e.t.c.The antidiabetic plants include Aloe vera, American ginseng and Blond psyllium and several other herbs. Aloe vera is a member of the Lily family of which there are over 240 species but only four are recognized as having hypoglycemic value with Aloe barbadensis leading. Preliminary research suggests aloe gel might lower glucose levels. Dose is usually 50mg per day(Shane-McWhorler,2006).American ginseng is a popular Chinese medicine and recent studies have shown that it reduces blood glucose levels in Type ii diabetics.

Ginsengosides and possibly other constituents are thought to reduce postprandial glucose levels. Blond psyllium seed husk orally seems to significantly reduce postprandial serum glucose in patients with Type ii diabetes. It reduces glucose levels by 14-20 %(Basch, 2003).

1.1 PROBLEMS ASSOCIATED WITH HERBAL PREPARATIONS

Most often, herbalists diagnose disease without understating the pathogenesis of the disease hence they tend to treat the symptom rather than the ailments. Different tribes use different herbs for treatments of diseases (Oliver – Bearer, 1986). According to literature reports (Derbyshine, 2000), Aloe vera is used to induce abortion in Argentina, to treat constipation in Bolivia, for diabetes in Canary Islands, Stomach ulcer in panama, Asthma in Peru, piles in Saudi Arabia, as a contraceptive in South Korea, for hepatitis in Taiwan and prevention of syphilis in West Indies. It is also true that superstition may also play role in local herbal practice. For this reason, purgatives, diuretics e.t.c are often used in the belief that they may eliminate evil influence or disease (Tagbato and Townon, 2001). There may be major difficulties in isolation pure compounds from complex mixtures of substances often found in extracts. Also a problem is the issue of property rights often found amongst some indigenous populations where new plants are sourced for drug development (Okunyi *et a.l*, 2000).

Ethnobotanical information despite the problem associated with use of herbs indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes. The hypoglycemic activities of a large number of these plants have been evaluated and confirmed in different animal model (Onoagbe, 1999).

Modern science and technology could investigate how to maximize and standardize the affectivity of plant products. The active principles should be established and the plant organ be identified.

There is increasing number of hypoglycemic agents or drugs e.g sulfonylureas, bignanides, new insulin analogs, inhibitors of carbohydrate absorption, triplitazones, harmones and amylin. All these are sourced from plants or synthesized. Although some found to be effective, they posses high rates of secondary failure as a result of side effects associated with them. Thus, research interest has focused on various plants that posses hypolipidemic, anti-humor, antihyperglycemic and immune stimulating properties that may help in reducing the risk of various diseases.

1.2 JUSTIFICATION, AIMS AND OBJECTIVES OF THE RESEARCH

1.2.1 Justification

Diabetes mellitus is a common metabolic disorder which is frequently misunderstood and often not treated optimally. It is a large and continuously growing health problems worldwide associated with high rates of hospitalization and a high incidence of heart disease, angina, myocardial infarction and blindness. The orthodox drugs presently in use are found to posses numerous side-effects which made them not too safe for therapeutic application hence the need for alternative medicine with little or no side effects. Also the orthodox drugs are relatively expensive, which makes them unaffordable to most patients. These factors collectively neccessitate research into alternative means of managing the disease.

1.2.2 Aims

i. Collection and extraction of appropriate plants for research

ii. Preliminary phytochemcial screening of plant extracts

iii. Determination of safe doses and subsequently LD_{50} of crude extracts.

iv. Anti diabetic screening of crude plant extracts in alloxan – induced diabetic albino rats.

v. Bioassay guided fractionation of plant extracts in diabetic rats

vi. Spectral analyses (GC – MS, IR e.t.c) and structural elucidation of active components

vii. Toxicological screening of crude plant extracts viz: Acute toxicity and chronic toxicity.

1.2.3 Objectives

i. Verification of claims by practitioners and some literature reports on hypoglycemic effects of plants under investigation

ii To obtain plant extracts that could possibly be used as ethnomedicine or antidiabetic remedy.

iii. Identification of compounds from plant extracts for likely drug development.

CHAPTER TWO

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes mellitus is a generalized, chronic metabolic disorder manifesting itself in its fully developed form by hyperglycemia, glycosuria, increased protein breakdown, ketosis and acidosis (Boundy and Rosenberg, 1974). If the disease is prolonged, it is usually complicated by degenerative changes of the blood vessels, particularly in the retina, renal glomerulus and nervous system. In many respect, the metabolic abnormality resembles that produced in experimental animals deprived of insulin and by extension, abnormalities of insulin metabolism are usually present in human spontaneous diabetics.

2.2 HISTORY OF DIABETES:

The disease is undoubtedly of ancient origin. The first systematic description was written by Areteus of Capadocia In Asia Minor probably in the first century A.D. He described the excessive thirst and constant need to urinate, dry mouth, parched skin, loss of weight and interpreted the disease as "a melting down of the flesh into the urine". The ancient Hindu Vedas however associated the sweet taste of urine with glucose in the eighteenth century. Thus, the Greek word "Mellitus" was first used (Nostrand, 1979).

Minkowski and Von Mering in 1889 removed the pancreas from cereal dogs in an effort to find out if the gland was an essential entity of life. The researchers were surprised to find that flies were attracted to the canine urine. The urine was analyzed and found that urine had high levels of glucose. Thus, the first recorded

association between pancreas and diabetes. The search for an antidiabetic substance, presumed to be secreted by pancreas commenced about 1909 and the substance was discovered and identified to be insulin.

Early attempts to treat pancreatomized dogs with crude pancreas extract given orally to the canines proved unsuccessful, and the failure was later explained on the basis that the extract (A protein) was destroyed by protein cleaning enzymes in the gastrointestinal tract. Fredrick Banting and Charles Best in 1922 succeeded in preparing a pancreatic extract which when injected into diabetic patient or experimental animals was capable of lowering the blood sugar. This experiment led within a year to the use of insulin extract in human subjects, a practice which with some improvements continued to prevail in the treatments of diabetes today. The experiment was highly publicized and for a while it was believed that a "cure' for diabetes mellitus has been achieved. The administration of insulin did ameliorate the primary symptoms of the disease, including death and coma, but several years later, it was found that insulin did not cure the many serous long-term complications associated with the disease (Kolata, 1979).

Since the discovery of insulin, numerous substances, natural and synthetic have been investigated for their blood sugar lowering effects. Although, most of these drugs necessarily resemble insulin in their mechanism of action, yet some of them have certain beneficial effects on certain types of diabetes. Plants and plant products are also know to have certain antidiabetic effects. In this respect, Best and Scoff in 1923 demonstrated an insulin like activity in extracts prepared from potatoes, wheat, beef rot and celery. Hippocrates in 1938 noticed that immature green pods contain a substance having an effect similar to insulin which is

experimental animals lowered the blood sugar and weakened the hyperglycemic conditions following the administration of dextrose and adrenalin. He also noticed that a diet of bean pods reduced the risk of glyycosuria, acetonia of diabetic patients.

Although diabetes has been recognized since antiquity and treatments of various efficacy have been known in various regions since the middle ages, pathogenesis of diabetes has only been understood experimentally at about 1900 (Partlak, 2002).

In 1910, Sir Edward Albert Sharpey – Schafer suggested that people with diabetes were deficient in a single chemical that was normally produced by the pancreases – he proposed calling this substance insulin meaning island, in reference to the insulin-producing islets of langerhans in the pancreas (Partlak, 2002). The endocrine role of the pancreas in metabolism and indeed the existence of insulin was further clarified (Banting *et al*, 1922). Bets and colleagues went on to purify the hormone insulin from bovine pancreases which leads to the availability of an effective treatment-insulin injections.

Other landmark discoveries include; the identification of the first of the sulphonylurease in 1942, re-introduction of the use of biguanides in the late 1950s, introduction of metformin in 1979 to replace phenformin withdrawn world wide in 1977, the determination of the amino acids sequence of insulin by Fredrick Sanger, the radioimmuniassay (RIA) for insulin by Yalau and Berson in 1960, identification of the first thiazolidinediones as an effective insulin sensitizer in 1900s, and demonstration that intensive glycemic control reduces chronic side effects more as glucose levels approach normal (DCCTRG, 1993).

2.3 CLASSIFICATION OF DIABETES

It is quite clear that diabetes is not a single disease, but a syndrome which may be produced by a number of different factors. It is common practice to generally classify diabetes into two major groups viz: Diabetes mellitus and Diabetes Insipidus. The World Health Organization recognizes three main forms of diabetes mellitus namely: Type 1, Type 2 and Gestational Diabetes (WHO, 1999). Type 1 diabetes Mellitus is also referred to as juvenile –onset diabetes while type 2 is referred to as adult- onset or maturity-onset diabetes. Recently, a third type has been identified which was formally referred to as "Other types". Gestational diabetes is similar to type 2 in that it involves insulin resistance which a times disappears with child delivery.

2.3.1 TYPE 1 DIABETES MELLITUS (INSULIN DEPENDENT DIABETES MELLITUS, IDDM)

Type 1 diabetes mellitus or juvenile-onset diabetes is characterized by loss or destruction of the insulin-producing beta cells of the islets of langerhans in the pancreas, leading to a total deficiency of insulin. The main cause of this beta cell loss is a T-cell mediated autoimmune attack (Rother, 2007). There is no known preventive measure that can be taken against type 1, diabetes which accounts to about 10% of diabetes mellitus cases in North America and Europe (though it varies by geographical location). Most affect people are otherwise healthy and of normal weight when onset occurs. They sensitivity and responsiveness to insulin are usually normal, especially in the early stage. Type 1 diabetes can also affect both

children and adults but was traditionally termed "juvenile diabetes" because it represents a majority of cases of diabetes affecting children.

2.3.2 TYPE 2 DIABETES MELLITUS (NON INSULIN DEPENDENT DIABETES MELLITUS, NIDDM).

Type 2 diabetes mellitus arises due to insulin resistance or reduced insulin sensitivity, combined with reduced insulin secretion. The defective responsiveness of body tissues to insulin almost certainly involves the insulin receptor in cell membranes. In the early stage, the predominant abnormality is reduced insulin sensitivity, characterized by elevated levels of insulin in the blood. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver. As the disease progresses, the impairment of insulin worsens and therapeutic replacement of insulin often becomes necessary. In the last decade, type 2, diabetes has increasingly begun to affect children and adolescents probably due to increased prevalence of childhood obesity observed in some place, (Arlam, 2003).

Type 2 diabetes may go unnoticed for years because visible symptoms are typically mild, non-existent or sporadic, and usually are no ketoacidotic episodes. However, severe long-term complications can result from unnoticed type 2 diabetes, including renal failure due to kidney damage, vascular disease, vision damage, loss of sensation or pain due to diabetic neuropathy, and, and liver damage from non-alcoholic steatohepatitis.

2.3.3 GESTATIONAL DIABETES

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2%-5% of all pregnancies and may improve or disappear after delivery. About 20%-50% of affected women develop type 2 diabetes later in life. Although, gestational diabetes may be transient, if untreated, the health of the foetus or the mother may be terribly affected. Risks to the baby may include macrosomia (high birth weight), congenital cardiac and central nervous system abnormalities, and skeletal muscle malformations. Increased foetal insulin may inhibit, foetal surfactant production and cause respiratory distress syndrome. Hyperbilirubinemia may result from red lood cell destruction. In severe cases, perinotal death may occur most commonly as a result of poor placental profusion due to vascular impairment. Atimes induction may be indicated with decreased placental function. A cesarean section may be performed if there is marked foetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia (Baron, 1982).

2.3.4 TYPE 3 DIABETES

There are several rare cases of diabetes mellitus that do not fit into type 1, type 2 or GDM but accounts for up to 5% of all cases of diabetes. They are recently termed type 3 diabetes mellitus (Type 3A-3E). In the past, attempts to classify them became controversial. Type 3A may arise as a result of genetic defect in beta cells while 3B may be due to generally related insulin resistance type 3C and 3D may

also arise due to diseases of the pancreas and thermonal defects respectively. Type 3E may be induced by chemicals or drugs (DCCTRG, 1993).

2.3.5 DIABETES INSIPIDUS

This is a condition in which large amounts of very dilute urine sometimes as much as 25 litres per day is secreted. It is entirely unrelated to diabetes mellitus. It may be sub-classified as primary, secondary and nephrogenic. Both secondary and primary diabetes insipidus results from failure of the post-posterior lobe of the pituitary gland secret anti diuretic hormone (ADH), which encourages the reabsorption of water by the kidney tubule. In nephrogenic diabetes insipidus, adequate ADH is present but the kidney tubule does not respond to its signal to reabsorb water from the urine. Nephrogenic diabetes – insipidus is a sex-linked recessive disorder. All forms of the disease are characterized by extreme polyuria and polydipsia but no polyphagia (Alice, 1997).

2.4 AETIOLOGY OF DIABETES

The cause of diabetes mellitus is unknown, but heredity and diet are believed to play a major role in its development. Diabetes results when the pancreas produces insufficient amounts of insulin to meet the body's needs. It may also arise when the pancreas produces insulin but the cells are unable to efficiently use it (insulin resistance). The excess sugar remains in the blood and its subsequently removed by the kidneys (Edell, 2001). There is at present no universally accepted explanation of the cause for spontaneous diabetes but a variety of factors have been implicated as being of causal importance in the development of diabetes. These include: Heredity, virus infections, diet, immunological damages etc.

2.4.1 HEREDITY:

Heredity plays a prominent role in determining one's risk of diabetes, but only as a predisposing factor and not an absolute determinant. Genetic factors play a much more important role in the Type 2 than the Type 1 diabetes e.g the identical twin of a person who develops Type 2 diabetes after the age of 40 is almost 100% certain to develop the disease, but someone whose identical twin has the Type 1 diabetes runs only a 52% risk of also becoming diabetic. Parents with Type 1 diabetes face only a 2% risk of having a child with the disease, whereas the risk to the offspring of type 2 diabetic is about 10%. Genetic predisposition is apparently stronger in the case of Type 2 diabetes of the young with a 50% chance that the child of such a diabetic will also develop the disease (Harris *et al*, 1998).

2.4.2 VIRAL INFECTION

The evidence that viral infection might cause some forms of human insulin dependent diabetes mellitus is derived from epidemiological studies and isolated case reports. Studies in mice have shown that viruses can induce diabetes by two distinct pathogenic mehcnaisms. Destruction of pancreatic beta cells by direct cytolysis results form infection with the D variant of the EMC virus, Mengo virus 2T and coxsackie B4 virus, while induction of an autoimmune destructive process results form infection with reo virus Type 1 and rebella virus. The ability of virus, to induce diabetes in mice is dependent on the genetic background of the host as well as on the genetic make-up of the virus (Amer. Diabetes Assoc., 1997).

2.4.3 DIET

Dietary factors have been quoted as possible genesis for the rising incidence of insulin dependent diabetes mellitus in Northern Europe and North America. There are no direct available data relating diet after weaning to the development of diabetes in genetically susceptible children. However, two reports have provided circumstantial evidence supporting the proposition that dietary factors may at least in certain circumstances influence the development of human diabetes mellitus. Thus, an unusually high incidence of diabetes in boys born in the month of October in Iceland has been linked to the high nitrosamine content of a smoked mutton traditionally consumed at Christmas. Subsequent experimental germ cells rather than by a direct effect on the pancreatic beta cells of the foetus. In the second report, anti-gliadin antibodies were reported in 54% of children at diagnosis of diabetes mellitus less than two years of age. In addition, studies using the spontaneously diabetic, insulin dependent rats suggest that certain components of the diet may be essential for the expression of clinical diabetes in diabetes prone animals. Wheat and milk protein have been shown to have the strongest diabetogenic effect and are evidently capable of triggering the string of events which results ultimately in destruction of pancreatic islet insulin-secreting cells (Amer. Nat. Diabetes, Data group, 1995).

2.4.4 IMMUNOLOGICAL DAMAGES:

Auto-immune reactions, wherein the body's immune defense system attacks its own pancreatic tissue as though it were a foreign substance, are also suggested as a cause for the beta cell destruction of the Type 1 diabetes. An immune

response may be conducted by T-lymphocytes or by soluble antibodies in the blood or by both. These reactions may arise spontaneously, or by secondary to a viral infection of the pancreas that leaves the islet tissue modified in someway for attack (Dagogo *et al*, 1997). It is also clear that in both man and animals with spontaneous insulin dependent diabetes, the immune system retains the capacity to recognize and destroy transplanted insulin secreting cells indefinitely. The information below summarizes the evidence that insulin dependent diabetes is a slow autoimmune disease.

- Special gene linked genetic predisposition
- Association with other autoimmune disorders.
- Circulating islet cell cytoplasmic and surface insulin auto-antibodies in new cases.
- Manonuclear cell infiltration of pancreatic islets resulting in selective destruction of insulin-secreting cells.
- Recurrence of insulin and selective destruction of insulin secreting cells in pancreas.

2.5 BIOCHEMISTRY OF INSULIN AND ITS SYNTHESIS

Insulin was discovered as the factor in the pancreas (islets of Langerhans) that can alleviate the hyperglycemia of depancreatized animals and human diabetes. It is a small globular protein (M.W = 5,700) first observed in extraction of porcine gastric mucosa. The hormone contains a high proportion of hydrophobic residues and readily associates in solution to form dimmers and under certain

conditions, higher polymers. Insulin consists of two chains of amino acids: The 'A' chain (acidic chain) and the 'B' chain (basic chain). The 'A' chain contains 21 amino acids while the 'B' chain contains 30 amino acids linked together by two disulphide bridges (White et al, 1978).

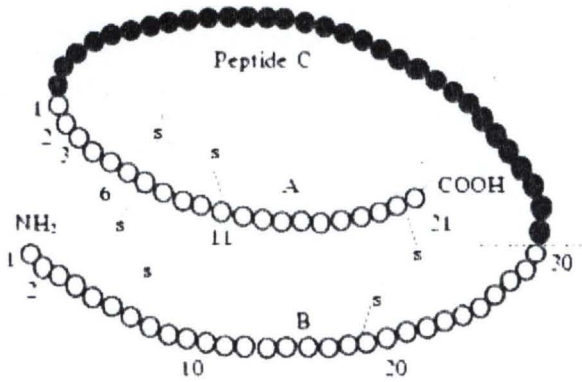


Fig 2.1. The structure of insulin.(Source:Suttie,1977)

2.5.1 SYNTHESIS:

The immediate precursor of insulin is a molecule named proinsulin. Proinsulin consists of insulin itself and a peptide loop that runs form the A chain to the B chain. The loop is referred to as connecting peptide or C-peptide. The final step involves the clipping of the C-peptide form the proinsulin molecule. The synthesis of pre and pro insulin occurs on the rough endoplasmic reticulum and the folding is accompanied by disulphide bond formation shortly after synthesis. The newly synthesized polypeptide is then transferred via an energy dependent process from the rough endoplasmic reticulum to the golgi apparatus at which site cleavage to

insulin begins (Makinson, 1980). :Synthesis of Insulin

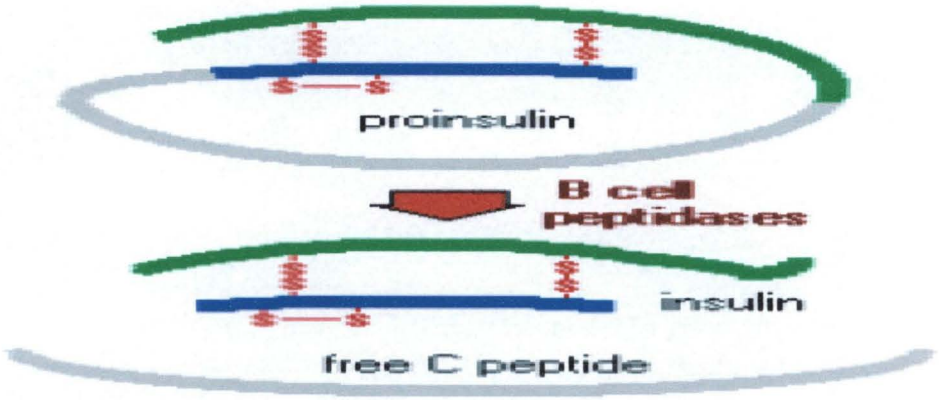


Fig2.2:Synthesis of Insulin (Source: Yudkin and Offord, 1980)

2.5.2 INSULIN SECRETION:

Insulin and the C-peptide are secreted in essential equimolar quantities and each is present in human serum. The secretion occurs at the surface of plasma membrane in an energy-dependent process in which the granule contents are liberated at the cell surface. The small amounts of proinsuin detectable in normal serum could lead to significant elevation of insulin in persons with normal pancreatic beta cells (White *et al.,* 1978). When the levels of glucose rise, the beta cells releases pulses of insulin which increases in frequency with increasing concentrations of glucose. These events are underpinned by cycles of change in electric potential across the cell membrane which in turn generates oscillations in the concentration of free calcium inside the cell. When the levels of calcium rise, insulin-containing granules move to the cell membrane and release their contents into the bloodstream. Under resting conditions, the membrane potential in beta cells is kept negative by potassium ion channels. These funnels through the plasma membrane allow potassium ions out of the cell. The exit of potassium ions allows the membrane

to become depolarized (becomes less negative) and voltage – dependent calcium channels open. In This manner, the potassium – ATP channels control the set-point of beta-cell electrical activity and their modulation has direct bearing on the regulated release of insulin (Mark, 1999).

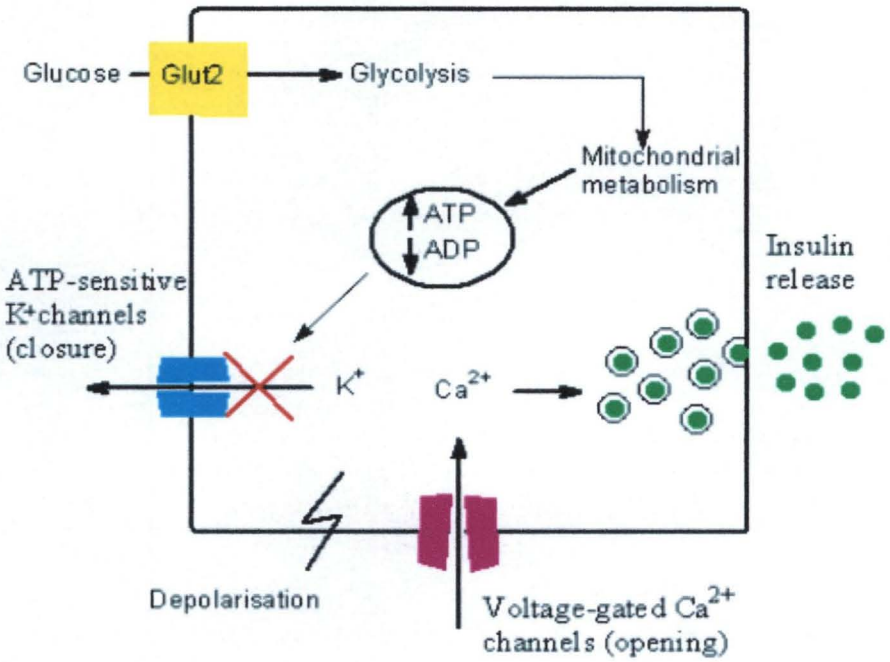


Fig 2.3: Mechanism of Insulin secretion (Source: Tierney *etal.*, 2002)

2.5.3 MECHANISM OF INSULIN ACTION

Insulin supplied to the blood occurs in a free state or as bound plasma proteins. Free insulin exerts influence on the metabolism of all insulin-sensitive tissues, while the bound insulin, acts on fat tissue only. Insulin sensitive tissues include muscular and connective tissues (fat tissue is a variety of connective tissue).

The liver is less sensitive to insulin; nervous tissues are also less sensitive to insulin too. Mammalian insulin receptors of glycoprotein nature have been found in tissues. These receptors a re numerous in the cells with a more pronounced susceptibility to metabolic insulin influence. The insulin-receptor complex is capable of drastically changing the cell membrane permeability for glucose, Amino acids, Ca^{2+}, K^{+}, and Na^{+} or to be more precise, of accelerating the transport of glucose, amino acids, K^{+} and Ca^{2+} into the cell. A major cause of this effect is a membrane-oriented action of insulin on active transport systems and the influence of this hormone on the generation of second messengers. At present, it is believed that the effects due to insulin are mediated by one or more peptide second messengers which activate CAMP phosphodiesterase and decrease CAMP concentration (Fajan, 1971).

These peptides stimulate the transport of Ca^{2+} and glucose. The most distinctive feature of insulin is its ability to intensify the active transport of glucose to the cells of hormone-sensitive tissues. The mechanism of insulin-stimulated glucose transport, into the cells is far from being clear. Presumably, insulin either directly interacts with the protein made up of glucose channels in the membrane and opens "pores for glucose passage" or directly through cyclic nucleotides which affects phosphorylation of membrane, proteins and thereby the membrane permeability for glucose. The activation of Na^{+}/K^{+} pump which leads to increased Na^{+}/K^{+} membrane gradient facilitation secondary active transport of amino acids into cell and fat tissues which presumably favours glucose transport too (Stroev, 1989).lower CAMP: CAMP ratio observed for insulin action facilitates glycogenesis, triglyceride and protein syntheses.

2.6 METABOLISM AND DIABETES

2.6.1 TYPE I DIABETES

In type 1 diabetes, because defective beta cell production of insulin, blood levels of insulin remain low in spite of elevated blood glucose levels. Even when dietary glucose is being delivered from gut, the insulin; glucagons ratio cannot increase, and the liver remains gluconeogenic and ketogenic. Since it is impossible to switch to the processes of glycolysis, glycogenesis and lipogenesis, the liver cannot properly buffer blood glucose levels. Indeed, since hepatic gluconeogenesis is a continuer's process, the liver contributes to hyperglycemia in the well fed state. The failure of some tissues, especially muscle, to take up glucose in the absence of insulin contributes further to hyperglycemic condition. Accelerated gluconeogenesis, fueled by substrate made available by protein degradation, maintain the hyperglycemia even in the starved state (NDDG, 1977).

It may seem an enigma that hypertriglyceridemia is characteristic of the condition, since fatty acid synthesis is greatly diminished in the diabetic state. However, the low insulin/glucagons ratio results in uncontrolled rates of lipolysis in the adipose tissues. This increases blood levels of fatty acids and results in accelerated ketone body production by the liver. If the ketone bodies are not used as rapidly as they are formed, ketoacidosis develops due to the accumulation of the ketone bodies and hydrogen ions. Not all the fatty acids taken up by the liver can be handled by the pathway of beta oxidation. The excess is esterified and directed into VLDL synthesis. The high levels of triglycerides observed in the bloodstream is because VLDL is synthesized and released by the liver more rapidly than those particles can b cleared from the blood by lipoprotein lipase. The quantity of this

enzyme is dependent on high insulin: glucagons ratio. The defect in lipoprotein lipase also results in hyperchylomicronemia, since lipoprotein lipase is also required for chylomycron catabolism in adipose tissue (Richard, *et al*; 1990).

The most important aspect of the diabetic state is that every tissue continue to play the catabolic role that it was designed to play in starvation, inspite of delivery of adequate or even excess fuel from the gut. The consequence is that metabolism becomes stuck in the starvation phase of the starve-feed cycle, with life threatening effects. Fig 2.4 below is a diagrammatic illustration of type 1 diabetes.

2.6.2 TYPE 2 DIABETES

In contrast to type 1, insulin is not absent but rather high levels of insulin may be observed and the protein is primarily insulin resistance rather than lack of insulin. Insulin resistance is a poorly understood phenomenon in which the tissues fail to respond to insulin. The number or affinity of insulin receptors is reduced in some patients; other have normal insulin binding, but abnormal post receptor responses such as glucose transport activation.

Obesity causes some degree of insulin resistance. Indeed, the majority of patients with type 2 diabetes are obese. Their insulin levels, which may be high, are not as high as those of a non-diabetic but similarly obese person. Hence, this form of diabetes is also a form of beta cell failure and exogenous insulin will reduce the hyperglycemia which results mainly because of poor uptake of glucose by tissues including muscles. In contrast to type 1, ketoacidosis does not develop because uncontrolled lipolysis in the adipose tissue is not a feature of this disease. On the other hand, hyperglyceridemia is a feature of type 2 but usually result from an increase in VLDL without hyperchylomicronemia. This is likely explained by rapid rates of de novo hepatic synthesis of fatty acids and VLDL rather than increased delivery of fatty acids from the adipose tissues (Na+, diabetes, data group, 1977). Fig 2.5 below is a diagrammatic illustration of type 2 diabetes.

2.7 DIAGNOSIS OF DIABETES

Diabetes is detected by:

a. A fasting plasma or serum glucose test which measures blood glucose after at least 8 hours without eating. The test is used to detect diabetes.

b. An oral glucose tolerance test which measures blood glucose after at least 8 hrs without eating and 2 hrs after drinking a glucose oral dose. This test can be used to diagnose diabetes or pre-diabetes.

c. In a random plasma or serum glucose test, the blood glucose level is determined in relation to when the last meal was taken.

In all cases, the positive results are confirmed by repeating the fasting plasma or serum glucose and oral glucose tolerance tests on a different day. Diagnosis is usually prompted by onset symptoms of excessive urination (polyuria) and excessive thirst (polydipsia) often accompanied by weight loss. These symptoms typically worsen over days to weeks. In some cases, physicians diagnose diabetes by administering glucose of a particular dose so as to measure the plasma or serum, glucose levels before and after (GTT-Glucose Tolerance Test)

Another test being developed for type 1 diabetes detects some specific "Antibodies" (proteins of the immune system that attack foreign substances) present only on person with diabetes. This test may detect type 1 diabetes at an early stage reducing the risk of complications from the disease (NIH, 2005). Table 1 shows the FPG tests and diagnosis.

The diagnosis of other types of diabetes is usually accomplished in other ways. These include health screening, detection of hyperglycemia during other medical

investigations; and secondary symptoms such as vision changes or unexplainable fatigue (WHO, 1999).

Table 2.1: Fasting Plasma Glucose Test (FPG)

Plasma Glucose (Mg/dl)	Diagnosis
99 and below	Normal
100 to 125	Pre-diabetes
126 and above	Diabetes

Confirmed by repeating the test on a different day (Source: NIH publications, January, 2005).

Most physicians prefer to measure a fasting blood glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance which takes two hours to complete.

2.8 COMPLICATIONS OF DIABETES

Diabetes mellitus of whatever type if left untreated may cause life threatening complications. Type 1 diabetes mellitus can result in diabetic coma (a state of unconsciousness caused by extremely high levels of glucose in the blood) or death. In both type 1 and 2 diabetes mellitus, complications may include among others blindness, kidney failure and heart diseases, it is primarily these complications, not the risks of ketoacidosis that account for the heavy burden the disease inflicts on society and make it the third leading cause of death in the United States. The most common complications are:

2.8.1 RETINOPATHY

Diabetes are twenty-five times more likely to become blind than non-diabetics for two reasons. Firstly, they are strongly predisposed to develop cataracts probably because the excess glucose in their blood polymerizes and is deposited in the lens of the eye (Arky, 1979). Secondly, because of the growth of tiny and poor-quality new blood vessels in the retina as well as macula, the blood flow and oxygen supply are impaired. This can lead to severe vision loss or blindness (Weiss, 2006)

2.8.2 NEPHROPATHY

In long-standing diabetes, the smallest blood vessels of nephrons (the functional filtration units of the kidney) frequently suffer the same type of damage as the blood vessels of the retina; this condition is called diabetic nephropathy. Severe diabetic nephropathy commonly leads to kidney failure and approximately 50% of type 1 diabetes die of kidney failure within 25yrs of the onset of diabetes (Drash, 1979). Diabetes mellitus is the most common cause of adult kidney failure worldwide.

2.8.3 PREMATURE ATHEROSCLEROSIS

Atherosclerosis (often called atherosclerosis or hardening of the arteries) is a condition in which arteries become progressively occluded by accumulations of cholesterol containing plaque. Although, it is common in older non-diabetes, the diabetics tends to develop it earlier and in a more severe form, the most common effects of atherosclerosis are coronary artery diseases and stroke, which result when the blood supply to the heart muscle or the brain is obstructed by a plague

within an artery. Coronary artery disease is the leading cause of death in long-term diabetics accounting for 75% of fatalities. Also, because of atherosclerosis and restricted blood flow, diabetics are quite subjects to gangrene especially of the extremities (Kolata, 1979).

2.8.4 NEUROPATHY

Diabetic neuropathy symptoms may include muscle weakness, pain in the extremities, local paralysis, urinary incontinence, sexual impotence (erectile dysfunction) and sensation of cold, heat or tingling in various parts of the body (Richard *et al;* 1990)

Much evidence indicates that most of the complications of diabetes are caused by the great variations in blood glucose levels common in this disease. Even diabetics who take regular insulin injections exhibit wide fluctuations in their blood glucose levels in-marked contrast to the tightly controlled blood glucose levels observed in normal people. According to the prevalent hypothesis, the more a diabetic succeeds in controlling his or her blood glucose to a near-normal level, the less risk there will be of developing any complications of diabetes mellitus disease.

2.9 EPIDEMIOLOGY OF DIABETES MELLITUS

Diabetes mellitus is by far the most common of the endocrine disorders worldwide. It is widely distributed and the incidence of both types (IDDM and NIDDM) is on the increase throughout the world. However, the prevalence of both varies considerably in different parts of the world. This seems to be due to differences in both genetic environmental factors. Epidemiology studies of whole populations have shown that the distribution of blood glucose concentration is uni-

modal with no clear division between normal and abnormal values. Therefore, diagnostic criteria are arbitrary (Eugene, 2004). Population studies involving Pima Indians in Arizona and civil servants in Whitehall have shown that hyperglycemia represents an independents risks factor for the development of disease of small and large blood vessels respectively. The current diagnostic criteria for diabetics of hyperglycemia have been shown to be associated with a significantly increased risk of disability and death from vascular disease irrespective of the basic cause of hyperglycemia.

The prevalence of the disease in Britain is between 1% and 2% but almost 50% of diabetes worldwide are non insulin dependent diabetes mellitus. In Europe and North America, the ratio of NIDDM: IDDM is 7:3. Some of the populations experiencing an increased prevalence of diabetes in United States are similarly being affected in other countries. Thus, in Central and South America, Africa and parts of Asia, the prevalence rates are skyrocketing. It should not be surprising that this same issue is occurring not only in developed countries but increasingly within the developing world too (Eugene, 2004). The America Diabetes Association point out in the 2003 assessment of the National Centre for Chronic Disease Prevention and Health Promotion that 1 in 3 Americans born after 2000 will develop diabetes in their lifetime (Narayan, 2003).

In Nigeria, the problem of record keeping and assessment of diabetes mellitus did not allow for a smooth statistical records so as to know the prevalence of the disease but, because of the poor economic state of the economy, a lot of people suffer from this disease even though a large number do not go for diagnosis in the established hospitals.

2.10 TREATMENT OF DIABETES

Once diabetes is diagnosed, treatment consists of controlling the amount of glucose in the blood (normalization of glucose in blood) and preventing complications. The immediate goals of treatments are to stabilize the metabolism, restore normal body weight and eliminate the symptoms of high glucose. The long-term goals are to prolong life, improve the quality of life, relieve symptoms and prevent long-term complications through careful dietary management, weight control, medication, physical activity (exercise), self-testing and education (Bevier *et al;* 1995).

2.10.1 DIETARY MANAGEMENT

This is one of the most effective ways for treating or managing diabetes through meal planning. The meal planning involves choosing healthy foods, eating the right amount of food, and meals at the right time. The American Diabetes Association and American Dietetic Association developed six food exchange lists for the purpose of people with diabetes. The lists include: starch or bread, meat and substitutes, vegetables, fruits, milk or diary and fat, every food has approximately the same amount of carbohydrates fat, protein and calories for the recommended daily intake. Any food on the list can be exchanged for any other food on the same list. The exchange list also show the number of food choices that can be eaten at each meal. Using the foods on the list, the distribution of calories can be controlled throughout the day so that food and insulin function will be balanced (Encyclopedia of Diabetes, 2001).

Meal plans differ depending on the type of diabetes. Within IDDM (Type 1), consistency in the time of meals eaten and the amounts, types of food intake is important to allow food and insulin drug to work so as to regulate the blood glucose levels. It meals and insulin are out of balance, extreme variations in blood glucose can well-balanced diet (Edell, 2001).

2.10.1.1 CARBOHYDRATE

People with Type 1 diabetes, because they experience absolute insulin deficiency, must use insulin to control glucose levels after meals. Since 1994, the American Diabetes Association (ADA) has recommended that, 60-70% of total calories come from carbohydrate and mono-unsaturated fat. Similarly, patients on fixed doses of insulin should attempt to keep the amount of carbohydrate relatively constant from meal (Rabasa-Lhoret, 1999; Fajan, 1971). Recommendations for carbohydrates consumption for people with Type 2 diabetes are similar to those with type 1. however there is some concern that increased unsaturated fat consumption may promote weight gain in obese patients with type 2 and thereby decrease insulin insensitivity (Frnaz et al; 2004). Therefore, even addition of fructose to the diet as a sweetening agent is not recommended by ADA. However, foods that contain naturally occurring fructose, such as fruits do not need to be avoided (ADA, 2007).

2.10.1.2 PROTEIN

Although the majority of clinical focus on the management of diabetes carbohydrate metabolism, protein metabolism in the diabetic state or statu abnormal. Patients with type 2 diabetes exhibit a more negative nitroge

than individuals without diabetes. Protein degradation appears to be exacerbated by hyperglycemia and improved by controlling glucose level but along with insulin therapy (Gougeon, 1998). These studies suggest that the protein requirements for people with type 2 diabetes may be slightly greater than those for non-diabetic individuals (Franz et al; 2004). Patients with type 1 diabetes can convert amino acids into glucose depending on the level of insulinization, therefore, protein consumption may lead to hyperglycemia. Studies on patients with type 2 diabetes however, have demonstrated that protein consumption does not increase plasma glucose concentrations and that endogenous insulin release is, infact, stimulated by protein consumption (Gannon, 2001).

2.10.1.3 DIETARY FAT

Recommendations regarding fat in the diet of people with diabetes are similar to those for patients with coronary artery disease. Because saturated fats are the major dietary determinants of serum LDL levels, people with diabetes, should strive to keep saturated fat consumption to less than 7% of total daily calories and to minimize consumption to trans-fatty acids. Cholesterol consumption should be less than 200gm per day (ADA, 2007). Considerably attention and marketing has been focused on the macronutrient content of diets of diabetes. Recent studies revealed that a diet low in carbohydrate and high in fat and protein may yield greater weight loss than other diets in non-diabetic patients (Gurdner, 2007). Similar diets studied in diabetic patients have also suggested that a low –carbohydrate diet may produce similar weight loss than balanced diets.

2.10.2 MEDICATION

There are three main approaches available on medications for the management or treatment of diabetes mellitus viz: Insulin Therapy, Oral drug therapy and use of medicinal plants.

2.10.3 INSULIN THERAPY

Insulin is available in a variety of formulations. These preparations differ from one another with respect to time course of action, route of administration, concentration, source of insulin and degree of purity. There are six basic preparations of insulin: natural insulin and five modified insulin. The modified ones are slower in onset than native insulin and have longer durations of action. The time course of the modified insulin's has been extended by two processes viz: complexing insulin with a protein and altering the physical state of insulin itself (Richard, et al; 1990). When classified according to time course of action, the basic insulin preparations fall into three groups: rapid-acting, intermediate-acting and long-acting. Regular and semilente insulin's are rapid-acting preparations (6-16hrs) while isophane insulin suspension, lente insulin are intermediate acting insulin (24hrs). The long-acting insulin are protamine zinc insulin and ultralente insulin (36hrs) duration.

The principal treatment of type 1 diabetes, even from the earliest stage, is replacement of insulin combined with careful monitoring of blood glucose levels; using blood testing monitors. Without insulin, diabetic ketoacidosis can develop and may result in coma or death. In the of type 2, within shots are taken for a more

effective blood glucose levels since type 2 diabetic can synthesize insulin only that insulin will not be used effectively.

2.10.4 DRUG THERAPY

Medications to control blood sugar are pills usually taken once or twice per day and there are many new drugs on test. These medications generally work by preventing the body from releasing sugar into the bloodstream, when insulin is not working properly, more than required insulin is released into bloodstream and helping the clearance of glucose from bloodstream into the cell. Novel oral drugs such as sulfonylreas, bignanides, glycosidase inhibitors and thiazolidinediones (TZDs) are also in various stages of testing (Bailey, 1999). Recently the first insulin sensitizers were released (Evan and Rusan, 1999).

Recent studies into how insulin is released from pancreatic cells have led to the development of several new drugs that enhance this process. Some of these, like repaglinide, are already available with many others are still in development. The success of insulin sensitizers like the biguanides and thiazolidinediones have ed to the discovery of new type of insulin sensitizers that work slightly in different ways. The discovery that thiazolidinediones work by activating a protein called PPAR gamma (peroxisome proliferators-activated receptor gamma) has spurred several companies to develop drugs that activated PPAR gamma in different ways (Bailey, 1999).

A number of drug options are available for treating type 2 diabetic but are not effective for type 1 patients. They are:

a. Sulfonylurea

The sulfonylureas are derivatives of the sulfonamide antibiotics but lack antimicrobial activity. They are subdivided into first generation and second generation agents. The principal difference between the two groups lies with their potencies: the first generation drugs are much more effective than the second generation drugs. However, although differences in potency are large, such differences are of minimal clinical significance.

Tolbutamide is an example of first generation sulfonylurea. It produces its initial effects by simulating release of insulin from pancreas and is incapable of insulin synthesis. It is for this reason that tolbutamide is of no value to insulin dependent diabetes. With prolonged use, tolbutamide offers the additional benefits of enhancing cellular sensitivity to insulin. Although the mechanism of this effect is not known, one hypothesis is that chronic tolbutamide increase the number of insulin receptors (Shinkai, 1999).

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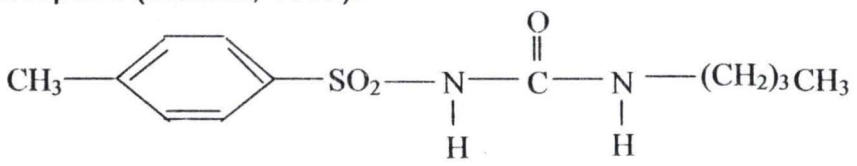
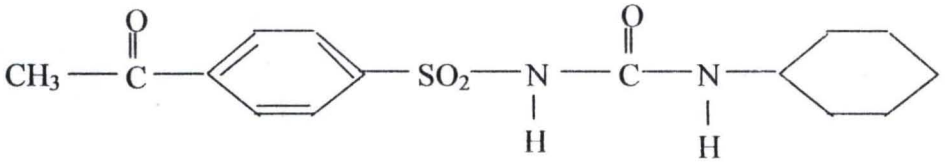
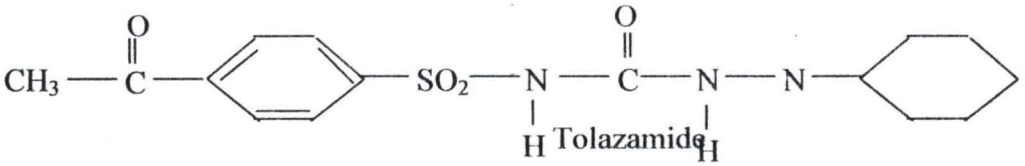


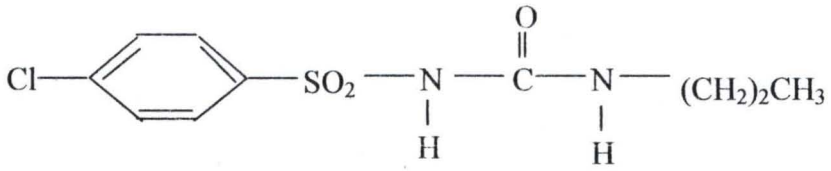
Tolbutamide (Orinase)
(1^{ST} Generation)



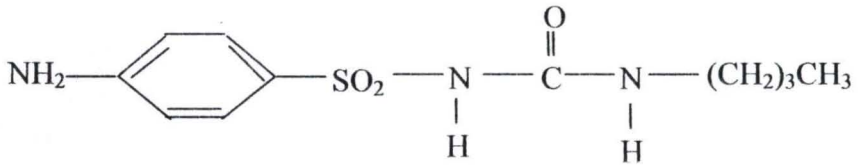
Acetohexamide (Dymelor) (1^{ST} Generation)



Tolazamide

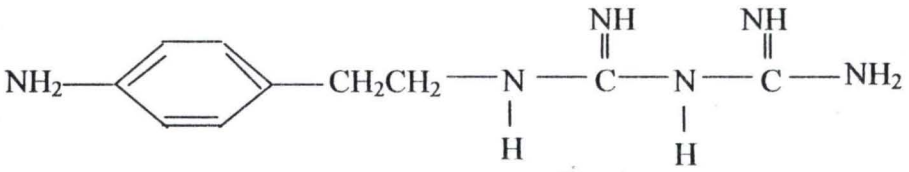


Chlorpropamide (Diabinese)



Carbutamide

b. Biguanides: metformin (Glucophage) and phenformin are in this category. They inhibit the production and release of glucose from liver. Their advantage is that they tend to cause less weight gain than other drugs. Side effects include a metallic taste in mouth, nansea, pain and diarrhea. Others include lactic acidosis and impaired kidney function (Shinkai, 1999).



Phenformin

c. Alpha-glycosidase inhibitors: These drugs blocks the action of enzymes in the digestive tract that breakdown carbohydrates hence sugar is absorbed slowly

into bloodstream. Drugs in this category include acarbose, miglitol. Side effects include diarrhea and liver damage (Vinik, *et al;* 2004).

d. Thiazolidinediones (TZD)

These drugs make the body tissues more sensitive to insulin and prevent liver from overproducing glucose. Side effects include swelling, weight gain and fatigue. A far more serious potential side-effect includes diarrhea and liver damage (Vinik *et al*; 2004).

2.10.4.1 DRUG COMBINATION

Drugs from different classes are combined to effectively control the blood sugar levels. In this case, newer medications such as Glucovance were developed. The drug contains both glyburide and metformin. Again towards the same direction, there is considerable interest in discovering drugs that could block the auto-immune responses. Such drugs are useful in the treatment as well as management of type 1 diabetes mellitus. The drugs are also useful in the control of auto-immune diseases such as multiple scterosis, rheumatoid arthritis and inpus. The principal goal of the drug action is to delay the progression of the full-blown disease (Rosen and Evan 2001).

2.10.5 SELF-TESTING APPROACH

Blood sugar testing or self-monitoring of blood glucose is done by checking the glucose content of a small drop of blood. The testing is done on a regular basis and will inform the person with diabetes how well diet, medication and physical activity are working together to control diabetes. The results can be used to adjust

meals, activities, or medication to keep blood-sugar levels within an appropriate range. Self-testing will identify high and low blood sugar levels before serious problems develop (Edell, 2001).

There are two methods of testing blood glucose measurements; one method is a visual comparison with small plastic strips. The second method is a meter test that provides a more exact reading of blood glucose. Ketone test is another test that is used more frequently in type 1 diabetes as well as type 2 diabetes during stress, illness or complications. The test is one on urine samples. Ketone test is performed in the following circumstances:

- When blood sugar is greater than 240mg/dl
- Illness
- Ketoacidosis
- Extreme stress
- Pregnancy (Edell, 2001).

2.11 OTHER APPROACHES TO DIABETES MANAGEMENT

Regular exercise especially is important for diabetes. It helps in controlling the amount of sugar in the blood and helps burn excess calories and fat to achieve optimal weight. Exercise improves overall health by improving blood flow and pressure. It also increases the energy level, lowers tension and improves the ability to handle stress. Some factors are taken into consideration for exercise:

- Choose an enjoyable physical activity that is appropriate for the current fitness level.

- Get involved in everyday physical activity
- Monitor blood glucose levels by home testing before and after exercise
- Carry food that contains sugar in case blood glucose levels get too low during or after exercise
- carry a diabetes identification and change for a phone call in case of an emergency
- drink extra fluids that do not contain sugar during and after exercise
- Changes in exercise intensity or duration to keep glucose levels within an appropriate range (Edell, 2001).

2.11.1 EDUCATION

Diabetes education is an important part of diabetes management. Diabetes educator and health care providers are available in many areas to teach essential skills needed after initial diagnosis of the disease. Appropriate education teaches people with diabetes how to incorporate the management principles of the disease into their daily lives an minimize dependence upon the health care provider. Basic principles called survival skills include:

- How to administer insulin or oral hypoglycemic drugs
- How to recognize and treat low and high blood sugar
- How to test and record blood glucose and urine ketones
- How to adjust insulin, food intake or both for changes in the usual exercise and eating habits
- How to handle sick days

- Where to buy diabetes supplies and how to store them

After considering the basic principles of diabetes care and a routine has been established (after several months), an education programme is helpful to learn more about the disease process, how to control and live with diabetes, and immediate and long-term complications of the disease. Annual review of diabetes information is strongly recommended. Continuous up-dating of personal knowledge of diabetes is encouraged because new research and improved ways to treat the disease are constantly being developed (Edell, 2001).

2.12 MEDICINAL PLANTS

There are many hundreds of medicinal plants that can be grown in both temperate and tropical climates and there can be probably a great deal more with properties yet undiscovered. A medicinal plant can be described as that plant either in part or whole contain substances that can be used for treatment purposes (Sofowora, 1982). Plants with medicinal properties are popularly referred to as the gift of nature to mankind. Medicinal plants are in use for centuries in the traditional system of medicine like Ayurveda in India and other countries for the treatment of diseases including type 1 and 2 diabetes mellitus. They are considered to be effective and relatively non-toxic and have vast potentials but are only partly explored by modern methods (Murphy, 1994).

Recently medicinal plants are used in the form of cold or hot aqueous extracts, soups and drinks. They accumulate in addition to providing the ecosystem with food, fuel and shelter, the chemicals as by-products of major metabolic pathways. Such components include: alkaloids, flavoroids, glycosides, volatile oils, saponins,

terpenes, and anthraquinones which among uses are key healing ingredients found in plants (Ghani, 1985). Some of the active principles in these plants have high hypoglycemic activities. Few of them are charatin from *Mormodica charantia* (bitter gourd), *Leucope largoidin* (Ficus bengateses) from bany a tree bark which are useful in the treatment of mild but less severe diabetes (Augusti, 1994). The fenugreek principles are highly potent and active in severe diabetes when tested on experimental animals (Murphy, 1995). In addition, they have hypocholesterolemic effects.

The actual figures numerically of medicinal plants in use have not bewen accurately determined but as at 1696, about 737 medicinal plants have been established in Dr. Solomon's dispensary (Shellard, 1979). It has also been discovered that one in four of all prescribed drugs and several prescriptions of such, are of plant origin (Lewington, 1990). With increased knowledge and advanced technology, more methods of extractions were developed and effectively utilized to isolate active principles fro medicinal plants for the treatment of diabetes. An international survey indicated that 33% of drugs manufactured in the developed countries are obtained from plants (UNCTAD/GATT, 1974). The dependence on herbs for cure have been progressively on increase as the orthodox drugs are costly and not easy to come by (Bodekar, 1997).

Surprisingly, the large quantity of modern drugs came from less than 155 plants which are known to be pharmacologically investigated out of an estimated 250,000 species of higher plants on earth (Farnsworth and Bingel, 1977). In view of the advantages credited to traditional herbal utilization, it is not surprising that research into medicinal plants have been an increase probably they are affordable when

compared to the orthodox drugs. Medicinal plants and drugs developed from them in most cases if not all have the potentials for active principles against minor problems such as cuts, brushes and major ones like coronary artery diseases, cancer and Aids (Hahm *et al;* 1982). Examples of such medicinal plants are: *Eugenia Jambolana,* fenugreek seeds which have been tried in human and found to be hypoglycemic (Murphy, 1995).

The chemical constituents of plants have various therapeutic potentials which are useful:

a. As template molecules, blueprints or precursors for the design and synthesis of now drugs

b. As tools to help in the explanation of the physiological and pharmacological mechanisms in drug development and testing

c. Inserting them directly into drugs and many medications. The chemical ingredients extracted from plants could be classified into anti-diabetic, anti-inflammatory, diuretics, contraceptives and laxatives (Sofowora, 1982).

Some of the extracts from plants are specific for a particular ailment while a lot of them require mobilizers, sensitizers or potentiation so as to work effectively. (Sofowora, 1982).

2.13 FUTURE THERAPEUTIC APPROACHES

It is clear that no method now available for treating diabetes really restores the metabolic pattern to normal. The precise control of metabolism provided by the interlocking secretion of insulin, glucagons, growth hormone, corticosteroids and catecholamine cannot be reproduced even roughly by the parental administration of

insulin or the oral hypoglycemic agents as they are now used. Recognition of the inadequacy of current treatment regimens with respect to the persistence of metabolic abnormalities as well as the increasing frequency of long-term complications of diabetes has led to a search for new approaches to treatment.

Current investigative efforts are directed at six possible modalities: use of implantable insulin pumps, insulin inhalers, pain free glucose tests, continuous monitoring device, islet cell transplantation and gene therapy (Rosen and Evan, 2001).

2.13.1 IMPLANTABLE INSULIN PUMP

Researchers are working hard to develop an implantable insulin pump that can measure sugar levels and deliver the exact amount of insulin required. This will make it possible to mimic the action of natural insulin delivery. However, the major problem remains the development of a glucose sensor which may be implanted under the skin. Other studies have shown that pre-programmed continuous infusion procedures may be effective in the absence of a glucose sensor. Most recently, normalization of blood glucose as been achieved in juvenile diabetes with a portable insulin infusion pump with which insulin is administered subcutaneously (Edell, 2001).

2.13.2 INSULIN INHALERS

Although daily insulin injection will still be needed, inhaled insulin is currently in clinical trails and these inhalers are about the size of a flash light and uses rapid

action insulin. The sprayed insulin passes quickly into the bloodstream to exert its effects (Edell,2001)

2.13.3 NEW INSULINS

In the past few years, three new formulations of insulin have become available which were designed to offer the advantages of simpler regimens and better glucose control for people whose cases must be treated absolutely with insulin. All are human insulin analogs derived and A 75/25 lispro mixture.

Glargine:- (from Averitis Co) is a basal insulin, offering a more continuous activity with much less of a peak than nautral insulin. It can be used with very rapid acting insulin such as lispro or aspart and should provide a flatter basal amount of insulin. Until now, this development has been possible with twice daily injections of ultra-lente or by the basal rate of insulin pump. Glargine application tries to permit a more normal meal-time patterns individualized to a persons own habit.

Aspart:- (from Nov Nordick Co) is a very rapid acting insulin that can be injection 15mins prior to eating. It is fast action also allows more freedom in the timing of meals and the amount of food eaten.

A 75/25 lispro mixture:- (from Eli Lilly Co) contains Lily's very rapid acting lispro and a novel human insulin analog called NPL. It is designed for those who need better control after meals and want to use an insulin pen. (Edell,2001)

2.13.4 PAIN FREE GLUCOSE TEST

The package contains both lancing device and a blood glucose meter all in one. It allows patients to monitor their blood sugar without the pain of sticking the

fingers to get blood samples. It was a unique disposable test strip to collect blood samples from forearm, thigh or upper arm areas that have fewer nerves ending such that it does not hurt as much as a finger stick (Rosen and Evan, 2001).

2.13.5 CONTINUOUS MONITORING DEVICE

A wristwatch like device was developed (Vygnus, Inc. U.S.A) that provides more information for managing diabetes. It is intended for use along with not as a replacement for finger stick blood test to monitor glucose, in order to ensure accurate results. The gluco-watch extracts fluid through the skin by sending out tiny electric currents. The wristwatch may be worn for 12 consecutive hours producing 3 measurements every hour even while asleep. An alarm will sound if the blood glucose levels are detected to be low or if a measurement was skipped as can occur in the presence of excessive sweat. The device detects trend and patterns in glucose levels in adults that are between the ages of 18 and above(Rosen and Evan, 2001).

2.13.6 ISLET CELL TRANSPLANTATION

Islet cells of the pancreas are the insulin-secreting cells. While whole pancreas transplantation continued to be used in certain circumstances, the shortage of suitable organs and the risks of lifelong immunosuppression (which leaves the patient vulnerable to serious infections) have prevented this procedure from being widely used. Attempts at pure islet cell transplantation in the past have been disappointing but a recent study has renewed excitement in this procedure. To

this end, several procedures for isolating, culturing and implanting islet cells have been worked out but improvements are still needed. Perhaps the most exciting technology of recent is the use of stem cells derived from pancreatic ducts. Stem cells are immature cells that can be renewed indefinitely and can be induce to form mature pancreatic beta cells. Thus, they would provide unlimited source of beta cells for transplantation. There have been recent successes with this technology in reversing the diabetes of mice with a form of type 1 diabetes. Although, experiments with humans is still been awaited (Rosen and Evan, 2001).

2.13.7 GENE THERAPY

Two recent reports describe research into gene therapy for different aspects of diabetes. These reports are in the fore front of what will no doubt be ongoing and exciting research from decoding of the human genome.

a. Scientists have identified a gene called ship 2 that appears to regulate insulin. Such findings make ship 2 a potential gene therapy target for the treatment of type 2 diabetes aimed at improving the individuals insulin regulation.

b. A protein that blocks the overgrowth of blood vessels in the eye is being studied as possible gene therapy for diabetic retinopathy. A recent study showed that treatment with the protein called pigment epithelium derived factor or PEDF prevented excessive new blood vessel formulation in an animal model. It may also be used to treat muscular degeneration (Rosen and Evan, 2001).

2.14 PREVENTION OF DIABETES

A number of studies have revealed that regular physical activities can significantly reduce the risk of developing type 2 diabetes. The studies also emphasized that simple and modest lifestyle changes could prevent diabetes. The following lifestyle changes have been encouraged in clinical trials to prevent diabetes.

- Chooses more vegetables, fruits, whole grains, low-fat dairy products and unsaturated fats.
- Eating healthy diet low in calories, fat and saturated fat i.e. limit fat intake to 30% of calories and saturated fat not more than 10% calories.
- Increasing fibre intake to 30g per day
- Reducing intake of sugar
- Engaging in moderate-intensity activities e.g. walking for at least 150mins each weak.

So far, clinical trails have proven that diabetes can be delayed for a period of at least six years. Studies conducted among diverse populations throughout the world absolutely demonstrated that type 2 can be prevented. Presently, the U. S. government is funding a nationwide study to see if type 1 diabetes can be prevented or delayed and more than 35 clinics and medical centres are taking part.

The study is operating on knowledge from smaller group studies that have shown that diabetes can be delayed by injecting regular small dose of insulin or taking oral insulin (Saundra and Christopher, 2005).

In response to the growing health burden of diabetes, the diabetic community has three choices: to prevent diabetes, cure diabetes, cure diabetes and take better care of people affected. Although prevention as well as cure remains elusive, all the three approaches are actively being pursued by U.S Department of Health and Human Services (Amer. of Diabetes Edu. 2000).

2.15 HERBS AND PHYTOCHEMICALS

Recently there has been an explosion of research concerning the health benefit of phytochemicals in herbs especially the medicinal herbs and it was concluded that, the higher the consumption of fruits and vegetables, the lower the risk of having any chronic disease such as cancer and diabetes mellitus. Conversely, those who ate few fruits and vegetables are more likely to die prematurely. Many consumable plants today are much poorer sources of potentially beneficial phytochemicals than the plant our Paleolithic gather ancestors consumed. Many phytochemicals which are biochemically active belongs to the group known as flavonoids, saponins, alkaloids, lignins and tannins. These compounds are usually bitter and so horticulturists have practiced negative selection for these compounds over the years (Duke, 1998). However, many of the phytochemicals are hypoglycemic agents. Many of such phytochemicals are available with us today as herbal medical remedies (Farnsworth, 1994).

2.15.1 EFFECTIVE HERBS FOR DIABETES

A number of plants are known to have anti-diabetic effects. About 1,123 herbs have been used to treat diabetes known to be hypoglycemic (Duke, 1998).

However, Marks and Farnsworth in 1994 caution that one third of these 1,123 plants may be dangerous. In all cases, these herbal products cannot be expected to control or reverse diabetes alone but are used as adjuncts to diet, and nutritional supplements. The plants are assembled according to their chemical constituents.

a. Plant with Phytosteringlycosides

The active constituent from the root bark of hypoglycemic ficus species (Ficus glomerate, F. religiosa) was found in India to be beta-sitosterol-D-glycoside, which shared hypoglycemic effect in fasting and alloxan-diabetic rabbits and in pituitary-diabetic rats comparable to that of tolbutamide. Bitter melon (*Momordica charantia* and *M. Foetida*) is used traditionally in India, Africa and Asia as diabetic remedy and has a bitter taste. Extensive investigations showed that in laboratory animals and gave good results in clinical trials. Bitter melon consists of a mixture of hypoglycemic compounds called "charantin" with an insulin like protein. The charatin appears to be a steroglycoside (physterin) but probably does not represent the whole activities of the fruit, as the favourable clinical results after daily use of 50-59ml of fresh juice could not be attributed entirely to few milligrams of charantin it contains. Charantin is more potent than tolbutamide in equivalent doses. The action of charantin was less pronounced in de-pancreatised cats but seemed to subsists in dictating the existence of sligh pancreatic activity (Duke, 1998).

The fruit of momordica foetida also contains hypoglycemic factor called "foetidin" which lowers the blood glucose of fasting rats. The action is comparable to that produced by a unit of insulin per kg. foetidin has been isolated and characterized as a chromatographically homogeneous product consisting of equal

parts of beta-sitosterol-D-glucoside and 5,25 stigmatadiene-I-glycoside (Olaniyi, 1975, Olaniyi and Marquis, 1975).

Phytosteroglycosides are also found in the leaves of morus alba and morus nigra together with anthocyanims. They are also found in the leaves of *Maytenus senegalensis* and seeds of *Asteracentha congifollo* which all have been shown to be hypoglycemic (Duke. 1988).

b. Plants with flavonoids

Flavonoids are frequently found in hypoglycemic plants (Harborne et al; 1974). The active constituent of vaccinum myrtillus leaves poured to be "neomyrtilin", a glycoside of 7-methly-delphinidin (Myrtillidin). The traditional use of an infusion of these plant leaves in diabetes in Europe was later justified by pharmacological and clinical trials showing that the effect of a single dose can last up to two weaks and more. The active principles allows gradual decrease of insulin dose in a number of patients. Leucodelphidin and leucocyanidin are found in flower and also in other parts of banana plants like *Musa sapientum*. Its pigment contains deoyxanthin-cyanidin (Hood and Laubury, 1954)

In evaluating the hypoglycemic effect of extracts or products of India plants, reputed to be anti-diabetic in native medicine, by the reduction of the normal fasting blood sugar-level in rabbits, Jain and Sharma found that an extract of the flowers of a variety of Musa sapientum was second in order of efficacy. A solution of 10mg/kg of the dried residue of this extract produced hypoglycemic of 15-24mg, compared with 20 to 30mg for *Allium cepa. Musa sapientum* extract flower was also more effective than *Syzygium cumini, Eycus glomerata, Mormordica charantin* and

Gymnema sylventre. Saponifiable and non-saponifiable fractions had hypoglycemic action (Mitra *et al*; 1975).

The leaves of *Morus alba* contain besides phytosterol glycosides, cyaniding and delphinidin glycosides. In another orally active hypoglycemic plant, *Syzygium cumin,* the fruits and seeds which are used as anti-diabetes contain cyaniding rhamnoglycosides, galli and elagi tannis respectively (Sofowora, 1982).

In the stem bark and root of *Rhizophora mucronata* (mangrove) also reputed to be hypoglycemic, catechol and tannis are found. Apart from anthocyanins, luecoanthocyanins, catechols and tannis, flavonoids are frequently found in hypoglycemic plants. *Thus querceneatin Kaempferol* and luteiolin glycosides are found in *Argyreia cuneata, Anacardium occidentale*, *Caiba pentandra, Centaurea porroti*, *Coccinea indica,lyceum barbarum*, *Olea europa*, *Phaseolus vulgaris*, *Phylanthus niruri* (also containing a luecoanthcyanin), *Scterocarya birrea* (flavonoids and tannins) and *Scoparia dulas.*

c. Plants with hypoglycemic organic sulphur compounds

Onions and Garlic (*Allium cepa* and *Allium sativum)*: as far back as 1923, Collip noticed a totally depancreatized dog could be kept alive for 66 days on three injections of crude onions extracts. Later a number of research workers confirmed that onion extracts have a distinct, slowly developing hypoglycemic action and that the effects are shown when he extract is given by mouth. Thus, the action of a petrol ether extract of sliced dried onion was equivalent to 62% of that of a tolbutamide (0.5g) standard dose and further purification led to an extract equivalent (76.6%) of tulbutamide standard (Matheu and Augusti, 1975).

Later, two active disulphides were isolated from fresh onions by steam distillation and solvent extraction. They were allyl propyl disulphide (APDS) and allian (diallyl disulphide oxide). The recently isolated principles from onions are carpacenes, thiosulphanate and zwiebelances. With APDS, blood glucose and glycosuria were significantly decreased in alloxan-diabetic rabbits and glucose tolerance was also improved. In a 4-hour test in fasting human subjects, APDS decreased the blood sugar (hourly controls) and also increased the serum insulin levels, while the free fatty acids levels remained the same. In contrast, in a control trial there was no fall in blood glucose but the serum insulin level decreased and the free fatty acids level increased considerately (Augusti *et al;* 1974, 1975).

The explanation for the outcome of the results was that insulin is a disulphide protein and its inactivation by compounds and albumins rich in SH-groups has been established. APDS probably removes insulin-inactivating compounds by competing with insulin for the thiol group in these compounds, thus producing an insulin-potentiating effect which prevents the increase of free fatty acids on fasting. In other words, the APDS bind to enzyme which serves to inactivate insulin thereby prolonging the life of an insulin molecule. In clinical trails, 100mg/kg allicin produced a significant drop in fasting glucose levels with a concomitant rise in serum insulin levels. The action in 12-subjects lasted for about 4 hours. Long-term feeding to normal rates of 100mg/kg of llicin produced a large reduction in lipid constituents of blood and liver and in this respect, allicin might have an advantage over tolbutamide (Augusti, 1975, 1976a and b).

Cyanidin and peonidin glycosides are also present in onion bulbs and could well be partly responsible for the effect of the crude extract. Garlic (*Allium sativa*),

Brassica oleraceae and even phaseolus al contain organic sulphur compounds. They posses anti-oxidants and lowers cholesterol levels significantly thus providing other benefits for diabetics (Duke, 1995)

d. Plants with Hypolycemic Alkaloids

Cathoranthus roseus (Madagascar Periwinkle) leaves are used for the treatment of diabetes, Catheranthus alkaloids extracted from leaves were administered in doses of 100mg/kg to rats with fasting hyperglycemia. Three of the alkaloids, vindoline and vindolinine, exerted at equivalent doses a more plant action. Only leurosine had a slight effect on cell division. In most cases, the hypoglycemic alkaloids are normally isolated from the other alkaloids before use since most alkaloids are cytotoxic (Maris and Jusey, 1976).

Hypoglycemic alkaloids are also found in *Tecona stans* (sometime, cultivated in West Africa). A hypoglycemic betain was isolated from *Trigonella foenum-graecum* and called trigonelline; it is a methyl-betaine of nicotine acid and was considered to the active constituent of fenugreek until 1974. When Isreali, researchers found that Coumarin and Nicotinic acid seemed to be the main hypoglycemic coustituent of all compounds isolated from the active fraction of the seeds. Trigonelline exerted a less pronounced but more persistent activity. The seeds also contain sterols (Shani *etal*, 1974).

Some active plants constituents contain nitrogen in amino groups (amanitins, hypoglycins, galegine). Unfortunately, toxicity or secondary effects exclude the clinical application of some of these plants with proven efficacy.

Blighia sapida is an indigeneous West African tree producing akee aple from which two hypoglycins A and B. These substances have strong hypoglycemic

and emetic action in most animals and non- Hypoglycin A proved twice as active as hypoglycin B. However the liver of animals treated by these substance showed fatty degeneration and reduced glycogen content. Hypoglycins appear to act through inhibition of the beta- oxidase enzymes, blocking the oxidation of long- chain fatty acids, thus causing accumulation of unmetabolized fatty acids making them unavailable for energy production. The organism reacts by oxidizing large amounts of glucose, thus causing a decrease of liver glycogen and a drop of glucose to hypoglycemic levels (Mitra, 1975).

The leaves of *Gymnema sylvestre* which are chewed in India to reduce glycosuria, normalize the blood sugar in diabetes subjects in about 3-4 weeks, but when used in combination with insulin, a prompt response was observed where insulin atoms in the particular dose had failed. The active principle is gymnemic acid and consist of a complex mixture of heterosides of diverse organic acids (Morris and Jusey, 1976; Mitra *etal*, 1975).

The majority of the plants have moderate action and can be useful in mild cases of diabetes, perhaps combined with a diet poor in anti diabetic agents. In some cases, their prolonged use might delay the establishment of a more serious diabetic disease. In a more severe cases, the plants can be used as an accessory treatment, making it possible to reduce the frequency and the dosage of insulin or other orthodox drugs/ the action of several plants is dependent on the presence of some insulin may be exogenous or endogenous.

2.15.2 MODE OF ACTION OF ANTIDIABETIC PRINCIPLES FROM HERBS

There are variety of ways to control the hyperglycemic syndrome. Generally, the antidiabetic mechanisms involved in hypoglycemic activity are numerous, including stimulation of insulin secretion, stimulation of glycogenesis and hepatic glycolysis, pancreatic beta cell potassium channel blockers, cAMP stimulation, modulation of glucose absorption from the gut(Marles and Farnsworth,1996). Apart from insulin and its substances have such effects e.g Somatostatin, Pituitary and Sex hormone, Corticosteriods and Prostaglandins. Therefore, the variety of hypoglycemic plants constituents and their diversity in action is mediated through the presence of Insulin. Such plants include: *Allium cepa*, *A. sativa*, *Corchorus olitorius*, *Syzygium cumini* and *Teconoma stans*. On the other hand, some plants also act in totally depancreatized animals. These includes *Blighia sapida*, *Galega officinalis*, *Ficus religiosa* and *Vaccinium myritillus*. Some plants extracts seems to remove insulin inactivating compounds through their thiol groups. Similarly, Incotinic acid present in some extracts acts as insulinase inhibitors (Peurat *etal,* 1966. 1967, 1978).

In a number of cases, some active principles in extracts seems to intervene in oxidoreduction phenomona while some act through inhibition of beta-oxidase enzyme systems. In this case, they block the oxidative enzyme of the Krebs cycle thus increasing anaerobic glycolysis and decreasing gluconeogenesis entailing an increased rate of glucose transfer from blood to tissues. Some extracts are believed to act by improving the vascularisation of the pancreas. Vascular complication arises gradually of the walls of small blood vessels and increases their permeability causing disturbance of metabolic exchanges. The

anthocyamide particularly inhibit or slow down these modifications of the disease. Thus, the improvement of diabetes achieved with some antidiabetic plants rich in anthocyanides could possibly be due to recovery of the vaxcularisation of the pancreas. Flavonoid found in some extracts also appear to act on capillaries in similar pattern (Pouratt, 1977). It thus appears that diabetic patients can be treated by a variety of plants intervening at different site of the glucose metabolism, and the success of the treatment may well depend on particular abnormalities of the individual cases. (Oliver, 1976).

2.15.3 PROMISING ANTI- DIABETIC HERBS

Investigations have been carried out on over 60 herbal extracts in a special cell culture to determine how much a particular compound stimulates the update and utilization of glucose. While these tests are not substitutes for human or animal studies, they are important because they identify safe compounds that act directly on the metabolism of cells. Plenty of plants and their phytochemicals can lower blood sugar levels but may accomplish it by imposing toxic effects on the body. From the investigations, Cinnamon was by far one of the most effective extract followed by Haxelgreen and black tea, from the extract of commercial cinnamon, new phytochemicals called dhalcone polymers were identified. These polymers increase glucose metabolism in the cell 20 folds or more. Chalcone polymers are also anti-oxidants that strongly inhibit the formation of reactive oxygen species in activated blood platelets. Thus they are also applicable to diabetic complications. The Goatsrue (*Galega officinallis*) has been tested in humans and was found very effective but still under toxicological

investigations. It contains a guanidine derivatives that is similar to the synthetic pharmaceutical hypoglycemic biguanide medications (Duke, 1998). While hypoglycemic herbs may offer promise in the treatment of diabetes in their combined effect with insulin, treatment is inherently disruptive a times and extreme caution must be exercise in order to promote a smooth transition, maintain suitable sugar levels. Table 2.2 shows some of the promising hypoglycemic herbs.

Table 2.2:Promising anti-diabetic herbs

Latin Name	Common Name	Use
Agrimonia pitosa	Hairy Agrimony	Hypoglycemic
Alisma plantageaquatica	Great water plantain	Hypoglycemic
Allium cepa	Onion	Hypoglycemic
Allium sativa	Garlic	Hypoglycemic
Allium cepaaggrefatum	Potato onion	Hypoglycemic
Allium cepaascalonicum	Shallot	Hypoglycemic
Allium cepaproliferum	Tree Onion	Hypoglycemic
Adiantum capillus-veveris	Adiantum plant	Hypoglycemic
Anacardium occidentale	Cashew leaves	Hypoglycemic
Andrographis paniculata	Kirata leaves	Hypoglycemic
Arctium lappa	Burdock roots	Hypoglycemic
Atriplex halimus	Salt Bush leaves	Hypoglycemic
Argyreia cuneata	River	Hypoglycemic
Anemarrheria asphodeloides	Zhi Mu	Hypoglycemic
Arctin minus	Lesser Burdock	Hypoglycemic
Astragalus membranaceuos	Huang Qi	Hypoglycemic
Astratylodes japonica	Japanese Atractylodes	Hypoglycemic
Bideus pilosa	Acestilla Plant	Hypoglycemic
Blighia sapida	Akee apple plant	Hypoglycemic

Brassica oleracia	Cabbage	Hypoglycemic
Cecropia optusitolia	Guarumo Leaves	Hypoglycemic
Coccima grandis	Coccinia roots	Hypoglycemic
Coccina indica	Ivy gourd	Hypoglycemic
Corchorus olitorius	Jute leaves	Hypoglycemic
Cautarea latifkra	Copalchi root	Hypoglycemic
Outarea sativus	Cucumber fruit	Hypoglycemic
Cumimum cyminium	Cumin Seed	Hypoglycemic
Cichorium intybus	Chicory	Hypoglycemic
Cirrsium ochrocentrum	Yellow thistle	Hypoglycemic
Coix lacrymajobu	Jobis Tears	Hypoglycemic
Cenyea canadensis	Canada Fleabane	Hypoglycemic
Cynara scolymus	Globe Artichoke	Hypoglycemic
Drosera rotundifolia	Sundew	Hypoglycemic
Elentherococcus senticosus	Siberian Ginseng	Hypoglycemic
Epimedium grandiflorum	Barrenwirt	Hypoglycemic
Eucalyptus globules	Tasmanian	Hypoglycemic
Euonymus alatus	Winged Spindle Tree	Hypoglycemic
Galega officinalis	Goats Rue	Hypoglycemic
Gynostemme peutaphyllum	Sweet Tea Vine	Hypoglycemic
Gymnema sylvsetre	Gymnena leaves	Hypoglycemic

Hordeum vilgare	Barley	Hypoglycemic
Hydrophila auriculata	Barleria Plant	Hypoglycemic
Hydrastis canadensis	Goldenseal root	Hypoglycemic
Honhuynia cordata	Tsi	Hypoglycemic
Inula helenuim	Elecanpane root	Hypoglycemic
Lablab purpureus	Hyacinth bean	Hypoglycemic
Lactuca sativa	Lettuce	Hypoglycemic
Latus comiculatus	Birds foot Trefoil	Hypoglycemic
Lotus albus	White Lupin	Hypoglycemic
Lycium barbarum	Box Thorn	Hypoglycemic
Lycium chinere	Chinere Boxthorn	Hypoglycemic
Lycopus virginicus	Bugleweed	Hypoglycemic
Lythrum salicaria	Purple Loose strife	Hypoglycemic
Morus alba	White mulberry	Hypoglycemic
Morus nigra	Black Mulberry	Hypoglycemic
Musa sapientum	Banana flowers & roots	Hypoglycemic
Nymphaea lotus	Lotus roots	Hypoglycemic
Nasturtium officinale	Watercress	Hypoglycemic
Nasturtium x sterile	Brown watercress	Hypoglycemic
Ocimium sanctum	Sacred basil plant	Hypoglycemic
Oleo europaea	Olive	Hypoglycemic
Opuntia spp	Pear stems and fruit	Hypoglycemic
Panax ginseng	Ginseng	Hypoglycemic

Panax pseuduginseng	San Q1	Hypoglycemic
Phaseolus vulgaris	French bean	Hypoglycemic
Phellodendran amurense	Amur cork tree	Hypoglycemic
Phellodendran chinese	Chinese cork tree	Hypoglycemic
Platycodan grandiflorus	Balloon flower	Hypoglycemic
Polygonatum odoratum	Solomon's seal	Hypoglycemic
Potentilla erecta	Tormentil	Hypoglycemic
Rehmania glutinosa	Chinese foxglove	Hypoglycemic
Rosa multiflora	Japanese rose	Hypoglycemic
Scoparia dulcis	Sweet brain leaves	Hypoglycemic
Syzygium jambolanum	Jambul seeds	Hypoglycemic
Spinacia oleracea	Spinach leaves	Hypoglycemic
Tecoma stans	Tronadora leaves	Hypoglycemic
Trigonella foenumgraecum	Fenugreek	Hypoglycemic
Triticum sativum	Wheat leaves	Hypoglycemic
Urtica pilulifera	Roamn nettle	Hypoglycemic
Urtica dioica	Stinging nettle	Hypoglycemic
Urtica urens	Annual nettle	Hypoglycemic
Vaccinium caepitosum	Dwarf bilberry	Hypoglycemic
Vaccinium membranaceum	Mountain huckleberry	Hypoglycemic
Vaccinium ovalifolium	Black huckleberry	Hypoglycemic

Vaccinium ovatum	Evergreen huckleberry	Hypoglycemic
Vaccinium parvifolium	Red bilberry	Hypoglycemic
Vaccinium scoparium	Grouse berry	Hypoglycemic
Vaccinium uliginosum	Bog bilberry	Hypoglycemic
Vitis vulpine	Frost grape	Hypoglycemic
Xanthium strumerium	Cackle bur	Hypoglycemic
Zea mays	Sweet corn	Hypoglycemic

Source: Who technical report series 727, Geneva, 1985

CHAPTER THREE

MATERIALS AND METHODS

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Collection

Six (6) plants used in this study enlisted in table 3.1 were variously obtained for screening from Kano, Minna, Maikunkele and Bosso. They were then identified by both: Department of Biological Sciences, Federal University of Technology, Minna and National Institute for Pharmaceutical Research and Development, Abuja, The scientific and local names as well as the parts used for the research are given in the table below:

Table 3.1 : List of Plants Analysed

Plant	Local Name			Part used
Scientific Names	Hausa	Yoruba	Igbo	
Zyzzipus spinachristi	Kurna	Ekannase-adie	Ogirili	Leaf
Artemisia herba-alba	Tazargade	Eemo	Akidi muo	Leaf
Terminalia glaucescens	Baushe	Idi Odan	Edo	Leaf
Moringa oleifera	Zogale	Ewe-ile	Okwe Oyibo	Leaf
Blighia sapida	Gwanja kusa	Isin	Okpu	Leaf
Anacardium occidentale	Yazawa	Kaju	Kausu	Leaf and stem bark

3.1.2 Experimental Animals

White albino rats (males and females) with average weights in the range of 80g and above were purchased from the National Veterinary Research Institute (NVRI) Vom, National Institute for Pharmaceutical Research and Development (NIPRD) Abuja and Faculty of Veterinary Medicine, (Ahmadu Bello University), Zaria. More of the animals were obtained through a pilot breeding scheme within the Department of Biochemistry, Federal University of Technology, Minna.

The rats were housed maintained in plastics cages with wire mesh in the Biology and Biochemistry Laboratories and were fed with pelletised broiler finisher feed (Grand Cereals and Oil Mills, Jos) and water ad-libitum during both acclamatization and experimental periods.

3.1.3 Chemicals and Reagents

Chemicals used were of analytical grade and obtained from reputable scientific and chemical companies such as: May and Baker Ltd, Sigma, Aldrich, GMBH Germany, Dagenham England, BDH Chemicals Ltd. The chemicals include Alloxan monohydrate (obtained from China) Dimethylsulfoxide (DMSO), hexane, ethylacetate, ethanol and chloroform.

Reagents

The reagents were of analytical grade and obtained from reputable companies such as: Agappe Diagnostics Ltd, Randox Laboratories Ltd, United Kingdom and Dialab Laboratories, Germany.

The reagents included: Cromatest triglyceride reagent (50mmol/L), PIPES buffer at PH 6.8, Lipoprotein Lipase ≥ 12u/l, Glycerokinase ≥ 10U/l, 20mmol/l of ATP, 40mmol/l of Mg^{2+}, peroxide ≥ 2.5U/l, 4-amino-antipyrine 0.5mmol/l, phenol 30mmol/l). Triglyceride standard (Glycerol 2.26mmol/L equivalent to 200mg/dl of glycerol troteate). Cromatest total protein reagent (contain cupric sulfate 6mmol/L, sodium potassium tartarate 21.0mmol/L, potassium iodide 6mmol/L, sodium hydroxide 0.75mmol/L) protein standard (Bovine serum albumin 7g/dL)./ glucose reagent (Glucose oxidase > 15u/ml, MOPS buffer 0.05mmol/L and phosphate buffer 0.025mmol/L and glucose standard 5.55mmol/L). Glucose kit, protein kit (Randox Laboratories Ltd, United Kingdom), Triglyceride kit, SGOT and SGPT kits (Agappe Diagnostics Ltd, Kerala, India) and Dialab laboratories, Germany). Erhlich's reagent.Others include creatinine and urea kit reagents.

Special Equipment

These included chromatography columns, GC-MS chromatographic instrument (GC 6890N and 5973 series MS selector). Infra-red spectrometer (SCHIMADZU FTIR Type). Spectrometer (spectronic 20D+): Hemocytometer.With the exception of spectrophotometer and hemocytometer, all others are at the Central Science Laboratory of Usmanu Danfodiyo University, Sokoto.

Methods

Reagents Preparations

Fehling's solution A and B

Solution A was prepared by weighing 17.32g of anhydrous copper I sulphate. It was then dissolved in 0.5M H_2SO_4 and made up to 250ml with distilled water.

Solution B was prepared by dissolving 86.5g of potassium sodium tartarate crystals in water. Distilled water was added to make up to 250ml mark in a volumetric flask. Equal volumes of solutions A and B were then mixed immediately and stored for use later.

- Bromine water

It was prepared by adding 3ml of bromine to 100ml of distilled water; the mixture was shaken occasionally and finally allowed to separate.

- Lead acetate solution

5g of lead II acetate was weighed and dissolved in 20ml of carbon dioxide free water. The PH of the solution was then adjusted to 7.5 with 1M sodium hydroxide. It was centrifuged and the clear solution was used.

- Mayer's reagent (potassiomeric cupric iodide solution)

1.34g of mercuric chloride was weighed and dissolved in 60ml of distilled water. 5g of potassium iodide was also dissolved in 10ml of water. The two were mixed and diluted with water to make up 100ml.

- Dragendroffs reagent: solution I was prepared by dissolving 17g of bismuth nitrate in 20ml of aqueous acetic acid. Solution II was prepared by dissolving 40g potassium iodide in water and made up to 100ml. Then distilled water the final solution composed of the mixture in the ratio 4:1:14 that is 21ml: 5.3ml: 73.7ml respectively.

- Wager's reagent: 10g of potassium iodide was added (dissolved) in distilled water. 2g of iodide was added to the solution, shaken and allowed to settle.
- Hager's reagent: 20g of picric acid was dissolved in 100ml ofwater
- Spray reagent for glycosides was prepared by adding 20ml of 10% phenylamine to ethanol acid then mixed with 100ml hydrochloric acid and 80ml acetic acid.

3.1.4 Preparation of Plant Extract

Aqueous plant extracts and organic solvent extracts

The appropriate plant organ or part (leaves, stem bark) were cleaned and dried to a constant weight at 25-30oCambient temperature. The dried materials were then crushed using mortar and pestle, and further blended using an electric blender.

45g of each plant materials were exhaustively refluxed with 400ml of the required distilled water or organic solvent for 2hours. The parent solutions were then filtered immediately with muslin cloth and the solvent evaporated over a water bath kept or maintained at below 40°C. Each of the semi-solid residues (extracts) were each weighed and percentage yield calculated thus:

$$\% \text{ yield} = \frac{\text{weight after extraction}}{\text{Weight before extraction}} \times 100$$

The same was repeated using the marc for the subsequent solvent extractions.

Weighed extracts were taken in labeled sample bottled and stored in labeled sample bottles and stored or kept in the refrigerator at 4°C until required for analysis.

3.2 Safe-dose (pre-LD50) determination

Preliminary acute toxicity of the crude plant extracts were tested in rats using different dose levels. Four rats (two male and two female) were taken for each dose level. The aqueous extracts were suspended in distilled water and administered orally (per-oral) using sterile apyrogenic disposable syringes to experimental rats. 0.9% normal saline was given to a set (4 rats) of animals to serve as control. The rats were observed for a period of one week (72 hours intervals) for clinical manifestations and mortality if any (Gamanmiel, 2000). Suitable doses were hence identified for each plant and used in subsequent.

3.3 Determination of Median Lethal Dose (LD50)

This was conducted using the Arithmetic method of Karber as adopted by Tyaniwura and Iliya (1989). Suitable doses of the plant extracts were selected for the acute toxicity tests (100mg/kg b.wt to 1,500mg/kg b. wt) while for the ethanolic extracts, the doses were 2000, 4000, 6000mg/kg b.wt.For aqueous extracts, test rats received a single dose of the plant extract while the control group received normal saline. For ethanolic extracts, the test rats received extracts suspended in 1% DMSO while the control received only 1%DMSO in distilled water.

Thereafter, the animals in both groups were observed for a period of one week and clinical symptoms and mortality recorded. At the end, The dosage at which half of the animals in each group died were recorded as median lethal dose.

3.4 Sub-Chronic Toxicity Tests

A pilot study was conducted by taking 3 rats each in 4 groups. Three of the groups are the test groups of single doses (2, 4, 6g/kg b.wt) while the remaining group is the control group. The animals were observed daily (i.e. 42days) and clinical findings recorded. Surviving animals at the end of experiment were sacrificed and histopathology performed.

3.5 Histopathology Method:(Akinsanya,2007)

The organs (liver,kidney,) were sliced and fixed in Boun fluid for 6-7hrs and later dehydrated by increasing concentrations of ethanol(70%,95% and then twice in absolute ethanol at 30mins duration). The tissues were then impregnated in molten paraffin wax three times and later allowed to solidify. The blocked tissues were sectioned at 4-5microns floated into precoated slides and dried. The sections were later stained with eosin stains. The stained tissues were then washed off in tap water and dried. They were later examined under the microscope and photomicrographs taken.

3.6 Chromatographical Methods

3.6.1 Thin-layer chromatography

40g of silica gel (thin layer gel 60 mesh size) was weighed into a conical flask and 80ml of distilled water added and shaken vigorously until a gel or a slurry mixture was obtained. Few grams of calcium sulphate were incorporated into the slurry in order to facilitate the adhesion of the adsorbent to the plates. The slurry was then poured onto a glass plate (cut into small sizes) placed on a spreader bed. The spreader was gently pulled across the plates evenly without interruption until all

the plates were uniformly coated with the silica gel. 0.25mm gel coating was used for analytical separations. The coated plates were allowed to dry at room temperature for 2hrs and then activated by placing them in an oven at 100°C.

Samples were applied to the coated glass plates by means of capillary tubes. The sample spotting were generally 2.5cm from the edge of the plates. The origin was marked before dipping into the thin-layer tank.

Plate development was carried out in glass tank which contained the predetermined solvent system (20mls of ethanol, 140mls of ethylacetate and 40mls of hexane – 1:7:2) to a depth of about 1.5cm. The tank was covered and chromatogram allowed to develop for 1hr and 30mins. At the end, the lid was removed and plates gently removed. They are dried at room temperature and subsequently introduced into the iodide tank for few minutes. The spots are then identified and marked.

3.6.2 Column Chromatography (Simple Type)

70g of column chromatography silica gel (60-120 mesh size) was transferred into 200ml of hexane in a beaker. Glass rod was used to facilitate the transfer and mixing to avoid air bubbles. Glass wool was placed at the bottom of the column. The column was packed by gently pouring the slurry into it with gentle tap adjustments to ensure that air bubbles are eliminated. The gel was added until the required height. The outlet was then opened until the gels had completely settled. A protective device (cotton wool) was placed n the surface of the gel to prevent disturbance during sample loading.

The sample (2.0g) was then carefully applied by pipette and allowed to run down the column. A small volume of hexane was then applied in a similar manner to wash final traces of the sample into the column.

Column development (separation) was achieved by stepwise elution in which solvents of varying polarities were used. The initial elution was carried out with a low polar solvent (Hexane, 200mls), followed closely with an intermediate polar solvent (ethylacetate, 200mls), then a mixture (1:1) of intermediate and highly polar solvents (ethylacetate/ethanol 200mls) and finally by a highly polar solvent (ethanol, 200mls). The resolved effluents from the column were collected in conical flasks for \subsequent analyses. The purified fractions were then subjected to spectral analyses.

3.7 Phytochemical Screening of Extract

This consisted of simple qualitative tests to detect the presence of alkaloids and other phytochemicals present in the extract. The screening procedures were those of Sofowora (1982), adapted from Wall et al; (1952 and 1954).

Tests for Alkaloids

0.5g of each extract was stirred with 5ml of 1% aqueous HCL on a steam bath. 1ml of the filtrate was treated with a few of Meyer's reagent and another 1ml portion was treated similarly with Drogendroff reagent. Turbidity or precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids in the extracts. (Harbourne, 1973; Trease and Evans, 1978).

A confirmatory test designed to remove non-alkaloid compounds capable of eliciting false positive reactions was carried out with all extracts which gave preliminary positive tests for alkaloids. A modified form of the thin layer chromatography method described by Farnsworth and Euler (1962) was used.1g of the extract was treated with 40% calcium hydroxide solution until the extract was distinctively alkaline to litmus paper, and then extracted twice with 10ml portions plates.

The chloroform extract was then spotted on thin layer plates. The plates were developed in a solvent system comprising of methanol, benzene, ethylacetate and chloroform in a ratio of 40:30:20:10 respectively. The presence of alkaloids in the developed chromatograms was detected by spraying with freshly prepared Drogendroffis spray reagent. A positive reaction on the chromatogram (indicated by an orange or darker colured spot against a pale yellow background) was confirmatory evidence that the plant extract contained an alkaloid.

Test for Indole Alkaloids

Dry extracts were dissolved in 2ml of 1% sulphuric acid, an equal volume of Erhlich's reagent was added slowly down the side for each tube and mixed. A hazy precipitate appeared indicating the presence of indole alkaloids (Trease and Evans, 1978).

Radulescu Test (Morphine Alkaloid)

Extracts residues were dissolved in 0.6ml portion of 1% sulphuric acid. 2ml of water, 2 drops of 10% nitrate solution were added and then ade alkaline with dilute ammonia. A deep brown precipitate indicates presence of morphine alkaloids.

Vitali mori test (Tropane alkaloid)

The extracts were dissolved in few drops of fuming nitric acid and evaporated to dryness and moistened with two drops of alcoholic potassium hydroxide solution. A purple solution indicates presence of tropane alkaloids.

Test for Caffeine (Urexide Test)

Plant extracts were treated with potassium chlorate and a drop of hydrochloric acid each. They were then evaporated to dryness and the residues exposed to ammonia. The presence of caffeine and other purine derivatives are given by purple coloured residues. This test further eliminates possible false-positive results for alkaloids (Trease and Evans, 1978).

Test for Saponins

The ability of saponins to produce frothing in aqueous solution and to homolyse red blood cells was used as screening tests for these compounds.

Froth Test

To 0.5g of each of the powdered samples, few drops of 95% ethanol was added to boiled. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30secs, then it was allowed to stand for over half an hour. A honey-comb froth is indicative of the presence of saponins (Wall et al; 1952, 1954).

To confirm the presence of saponins, 5.0g of each extract was boiled for 10mins with 50ml phosphate buffer, pH 7.4 and then allowed to cool and filtered; 5ml of the filtrate was passed for 3hrs through an asbestos disc (1.5mm thick and

about 7mm in diameter), which had been previously soaked with two or three drops of 1% cholesterol in either and dried. After filtration, the disc was washed with 0.5ml of distilled water, dried and boiled in 20l of oxylol for 2hrs to decompse the complex formed between cholesterol and any saponins in the extract. The disc was then washed in either, dried and placed on a 7% blood nutrient agar. Complete hemolysis of red blood cells around the disc after 6hrs was taken as further evidence of the presence of saponins.

Test for Tannins

3g of the portion of extract was boiled in 50ml of distilled water for 3mins on a hot plate. The mixture was filtered and the resulting filtrate was used to carry out the following:

Ferric chloride test: a portion of each of the extracts was diluted with distilled in a ratio o 1:4 and a few drops of 10% ferric chloride solution was added. A blue or green colour indicates the presence of tannis (Trease and Evans, 1978).

Ferric ammonium citrate test: o 1ml of each of the aqueous extract, 0.25% ammonium citrate solution was added to the mixture, sufficient solid sodium acetate was added to adjust the ph of the solution to 8 using PH indicator paper. This was boiled in a water bath and filtered. A coloured precipitted indicates the presence of tannis.

Test for Anthraquinones

Bontrager's tests: to show the presence of free anthraquinones, 0.5g of the extract was taken in dry test tubes and 10ml of chloroform was added and shaken for 5mins. The extract was filtered and equal volume of ammonia was added to the filtrate and again shaken. A bright pink colour in the upper aqueous layer indicates the presence of free anthraquinones (Trease and Evans, 1978).

For combined anthraquinones, 0.5g of extract was boiled with 10ml of 10% hydrochloric acid for 2mins. The extract was filtered. To the filtrate, equal volume of chloroform was added. The tube was inverted a couple of times avoiding vigorous shaking. The solution was transferred into a separating funnel and the two layers allowed to separate. The lower chloroform layer was poured into a clean test tube and 10% ammonia solution was added and shaken. The two layers were again allowed to separate. A bright pink colour in the upper aqueous layer indicates the presence of combined anthraquinones (Trease and Evans, 1978).

For anthraquinones derivatives, 0.5g of each extract was boiled with 10ml of 10% $Fecl_3$ and 5ml of 10% HCL for 5mins. The mixture was filtered and to the filtrate, equal volume of chloroform was added. The layers were allowed to separate in a separating funnel. The chloroform layer was transferred into another tube containing 5ml of 10% ammonia solution. A bright pink colour in the upper aqueous layers indicates the presence of anthraquinones.

General Test for Glycosides

1g of the extract was taken in solution and 0.2ml of dilute sulphuric acid was added. This was heated on a boiling water bath for 5mins, cooled and then

centrifuged. Each supernatant was pupated off and made neutral with sodium hydroxide solution. A drop each of Fehlings solution A and B were added and the mixture placed on a boiling water bath for two minutes. Brick-red precipitation was indicative of glycosides (Trease and Evans, 1978).

Tests for Cardiac Glycosides

Legal test

The extract was dissolved in pyridine and a few drop of 1% sodium nitroprusside together with a few drops of 20% NaOH were added. A deep red colour which faded to a brownish yellow indicated the presence of cardiac glycosides.

Kedde test

1ml of an 8% solution of the extract in methanol was mixed with 1ml of 2% 3,5-dinitobenzoic acid in methanol and 1ml of a 5.7% aqueous sodium hydroxide. An immediate violet colour indicated the presence of cardenolides in the extract. The colour fading gradually through reddish brown to brownish yellow with the precipitation of a whitish crystalline solid. This test indicated the presence of a lactone ring in the cardiac glycosides.

Keller-Killiani Test

0.5g of extract was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underplayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a

deoxy sugar characteristic of cardiac glycosides. A violet ring may appear below the brown ring and gradually spread throughout this layer (Trease and Evans, 1978).

Test for Cyanophoric Glycosides

2ml of the extract was taken in a test tube and a piece of sodium pirate paper suspended above the solution by trapping the top edge between the cork and the tube wall. This was allowed to stand for an hour. For a quicker reaction, a little sulphuric acid was added to the extract and heated gently. This resulted in the release of free hydrocyanic acid. A positive test would involve the conversion of sodium picrate (yellow) to sodium isopurpurate (brick red) Sofowora, (1982).

Lieberman's Test

0.5g of the extract was dissolved in 2ml of acetic anhydride and cooled. Sulphuric acid was then carefully added. A colour change from violet to blue green indicated the presence of a steroidal nucleus i.e aglycone (Shoppe, 1964).

Salkauski Test

0.5g of the extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interface indicated the presence of a steroidal ring i.e aglycone.

Tests for Volatile Oils

A small quantity of the extract was shaken with 0.1ml sodium hydroxide to which dilute hydrochloric acid was added. A white precipitate indicated volatile oils. In the second test, extracts were dissolved in 90% alcohol and drops of ferric

chloride solution were added. A green solution indicates presence of volatile oils (Trease and Evans, 1978).

Test for Resins

a. 4ml of the extract was taken and equal amounts of copper acetate solution were added while shaking and allowed to separate. A dark blue solution indicates resins.

b. 2ml of plant extract was mixed with equal volume of acetic anhydride solution to which some drops of concentrated sulphuric acid were added. A violet coloued solution indicates resins (Trease and Evans, 1978).

Tests for Flavonoids

The plant extracts were dissolved in water and magnesium chips were added. Drops of concentrated hydrochloric acid were poured down the side of the tubes. The presence of flavonoids was given by a brown precipitate. 1gm of plant extract was dissolved in sodium hydroxide solution. A yellow coloration that disappeared on addition of hydrochloric acid was also indicative of flavonoids. Plant extracts treated with. In chips and concentrated hydrochloric acid which produced some precipitate also indicates flavonoids (Trease and Evans, 1978).

Tests for Balsams

2 drops of alcoholic ferric chloride solution was added to 5ml of 90% ethanol extract of each of the extract. A dark green colour indicates the presence of balsams (Wall et al 1952).

3.8 INDUCTION OF DIABETES

Induction of diabetes was carried out by intraperitoneal injections of 200mg/kg b.wt Alloxan to animals fasted for 18hrs period. One week (7 days) after injection, the rats were fasted again and initial fasting blood glucose levels were determined. Animals that had the glucose level above 150mg/dl were selected for remaining 4 to 6 weeks duration of the experiment.

Blood samples were collected by Tail clip method (Alarcon-Aguilar *et al;*1999).

3.9 Hypoglycemic Activity of Crude Aqueous Extracts and stepwise fractions (p.o)

a. Administration of Aqueous Extracts (p.o): The rats were divided into 2 groups of 3 rats. Group IA consisted of rats induced but not treated and Group IIA consisted of rats induced and treated. Aqueous extracts (300,400,500,550,525,900mg/kgb.wt)ofAnacardium,Blighia,Moringa,Terminalia,Artemisia and Zizzypus leaves respectively were administered to GroupIIA while 0.9% normal saline administered to Group IA. The two groups were observed for a period of 4 weeks. The hypoglycemic activities were then recorded using lowering of blood glucose as parameter.

b. Administration of stepwise fractions of organic solvents (hexane, ethylacetate, and ethanol) p.o.Another set of rats divided into 3 groups of 3rats. Group IB consisted of normal rats (non-diabetic). Group IIB consisted of rats induced but not treated while Group IIIB consisted of rats

induced but treated. Group IB rats were admistered 1%DMSO only while Groups IIB and IIIB were administered fractions (200,300mg/kg b.wt) suspended in 1%DMSO. All the groups were observed for 4weeks.The hypoglycemic activities were then recorded using blood glucose lowering effect as a parameter.

3.10 Hypoglycemic activity of the Column Effluents (p.o).

Another set of rats divided into 7 groups of 3 rats each used. Groups IC and IIC consisted of rats administered (200,300mg/kg b.wt) ethyl acetate effluent. Group IIIC and IVC consisted of rats administered (200,300mg/kg b.wt) ethylacetate: ethanol effluents. Groups V c and VI c consisted of rats given (200,300mg/kg b.wt) ethanol effluent while Group VII c were given 500mg/kg bwt metformin as standard drug. All the effluents were suspended in 1%DMSO. The groups were observed for 4weeks. Hypoglycemic activities were recorded using blood glucose lowering effect as parameter.

3.11 Determination of Biochemical Parameters.

Glucose

Randox glucose (GOD/PAP-LIQUID) diagnostic kit (CAT/KAT. NR.GL 2623, GL 2614, GL2610) was used for the determination. The reaction principle is based on the enzymatic oxidation of glucose in the presence of glucose oxidase (GOD). The hydrogen peroxide formed reacts under catalysis of peroxidase (POD) with phenol and 4-amino phenazane to form a red-violet quinineimine derivative which is recorded at 500nm (Tietz, 1976).

$$\text{Glucose} + O_2 + H_2O \longrightarrow \text{glucoronic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazane} + \text{phenol} \longrightarrow \text{quinineimine} + 4H_2O$$

Procedure

1.0ml of the reagent was measured into the blank test tube, 1ml each of the reagents was pipetted into the standard and sample test tubes. To the sample tube, 0.01ml of the sample was added. All were mixed and incubated for 5mins at 370C.The absorbance of the standard and samples were measured against blank at 500nm.

$$\text{Glucose conc (mg/dl)} = \frac{\text{A sample}}{\text{A standard}} \times 100$$

Total Protein

This was determined using the RANDOX standard manual for invitro quantitative plasma/serum total protein diagnostic kit (Cat No TP245-Randox Laboratories Crumlin, U.K).

The principle is based on the interaction of cupric ions in alkaline media with protein peptide bonds resulting in the formation of a coloured complex read against a protein standard at 530nm (Tietz, 1995; Weichselbaum, 1992).

Procedure

1.0ml of the reagent was pipetted into a sample test tube and 0.02ml of the sample added. A standard reagent was prepared by adding 1.0ml of reagent was pipetted into blank tube. The resulting mixture was incubated for 10mins at 37°C. The absorbance was read at 540nm against blank.

Calculations:

$$\text{Total protein conc (g/L)} = \frac{\text{A sample}}{\text{A standard}} \times \text{conc. of standard}$$

Triglycerides

The Agappe Triglyceride kit (Cat Nos 1121500-11215004 Agappe Hills, Kerala, India) was used.

$$TGL + H_2O \xrightarrow{LPL} Glycerol + fatty\ acid$$

$$Glycerol + ATP \xrightarrow{LPL} Glycerol\text{-}3\text{-}p + ADP$$

$$Gylcerol\text{-}3\text{-}p + O2 \xrightarrow{GPO} Dihydroxyacetone\text{-}p + H2O2$$

$$2H_2O_2 + 4\text{-}aminoantipyrine + ADPS \xrightarrow{POD} Red\ quinine + 4H_2O$$

GPO: Glycerol-3-phosphate oxidaze

ADPS: N-ethyl-N-sulfopropyl-n-anisidine

LPL: Lipoprotein Lipase

GK: Glycerol kinase

The red quinone dye is read at 630nm (Buccolo and David, 1974; Wener *et al;* 1981; Annoni *et al,* 1982).

Procedure:

1.0ml of the reagent was measured into a test tube as blank; 1.0ml of the reagent was pipetted into a test tube containing 0.01ml of standard. 1.0ml of the reagent was pipetted into sample tube containing 0.01ml of serum. All were mixed and incubated for 5mins at 37°C. Absorbance was read at 520nm against blank.

Calculations:

$$\text{Triglyceride conc. (mg/dl)} = \frac{\text{A sample}}{\text{A standard}} \times 200$$

Glutamate pyruvate transaminase (SGPT or ALT)

GPT was determined using Agappe Diagnostic kit (Agappe Diagnostics Ltd, India, Cat Nos 11214001-11214004).

The test principle is based on the oxidation of NADH to NAD+, the resulting decrease in absorbance at 340 being directly proportional to the activity of GPT in the sample.

$$\text{L-Alanine} + \text{*-ketoglutarate} \xrightarrow[\text{GPT}]{\text{ALT}} \text{pyrurate} + \text{L-Glutamate}$$

$$\text{Pyrurate} + \text{NADH} + H^{+} \xrightarrow{\text{LDH}} \text{L-lactate} + NAD^{+}$$

ALT – Alanine aminotransferase

LDH- Lactate dehydrogenase

Procedure:

1.0ml of the reagent was pipetted into the tube marked standard and 1.0ml was also pipetted into blank tube. 1.0ml of the working reagent was pipetted and mixed with 0.1ml of the sample in the sample tube. They were mixed and incubated at 37oC for 1mins. Absorbances were read at 340nm.

Calculations:

$$\text{SGPT activity (V/L)} = \frac{\triangle \text{absorbance}}{\text{Mn}} \times 1768$$

Glutamate oxaloacetate transminase (SGOT or AST)

The enzyme was determined using Agappe Diagnostics kit (Agappe Diagnostic Ltd, india, Cat Nos 11214001-11214004).

The test using this kit is a modified formulation for the assay of GOT as recommended by the IFCC which involves adding pyridoxal phosphate at a level of 0.1mmol/L.

$$\text{L-ASP} + \text{2-oxoglutarate} \xrightarrow{\text{GOT}} \text{oxaloacetate} + \text{L.Glutamate}$$

$$\text{OAA} + \text{NADH} + \text{H+} \xrightarrow{\text{MDH}} \text{L-malate} + \text{NAD+}$$

AST – Aspartate Transaminase

MDH – Malate Dehydrogenase

NADH is oxidized to NAD+, the resulting decrease in absorbance at 340nm is directly proportional to the activity of GOT in the sample (Wolf, 1980).

Procedure:

1.0ml of the mixed reagents was pipetted into the marked standard tube and 1.0ml of reagent pipetted into blank. 1.0ml of working reagent was pipetted and mixed with 0.1ml of the sample. The tubes were incubated a 37°C and absorbances read at 340nm.

Calculations:

GOT(IU/L) = DA/min x 2134

Creatinine Determination

Principle:

Creatinine in the sample reacts with alkaline picrate to form a complex solution with a light yellow colour and the intensity of the colour is proportional to the amount of creatinine present in the sample. The absorbance is taken at 520nm.

Method

0.5ml of serum was pipetted into the labeled test tube and 1.5ml of distil-deionised water was added to all the labeled test tubes followed by 0.5ml of sodium tungstat and sulphuric acid and mixed.A milky precipitate was formed which was then centrifuge for 2mins. After centrifugation, 1.5ml of the supernatant was pipetted into another clean tube. 1.5ml of standard was pipetted into tube labeled standard and blank.0.5ml of NaOH and 0.5ml of picric acid was pipetted into each of the tubes. They were mixed and allowed to stand at room temperature for 20mins. The absorbance was read at 520nm.

Calculation

$$\text{Conc.} = \frac{\text{A sample}}{\text{A Std}} \times \text{Cons. Of Std. (mmol/l)}$$

Urea Determination

Principle

Urea is hydrolyzed to ammonia and carbon dioxide in the presence of urease.Ammonia reacts with oxoglutarate in the presence of NADH which is oxidized and measured at 340nm.

Method

2.0ml of distilled deionised water was pipetted into three labeled tubes, 0.02ml of serum and standard were pipetted into the labeled tubes respectively. 1.0ml of working reagent and mixed acid reagent were added to each of the labeled tubes and mixed. The tubes were placed in water bath for 20mins at 100°C. A

purple colour was produced which is proportional to the concentration of urea. The absorbance was read at 340nm.

Calculation

$$\text{Conc.} = \frac{\text{A sample}}{\text{A Std}} \times \text{Cons. Of Std. (mmol/l)}$$

3.12 Determination of Haematological Parameters

Packed Cell Volume (PCV)

Few drops of blood samples were collected from tail tip into the heparinized capillary tubes until its two third or three quarter filled with blood. One end of tube sealed with plasticine. Two hematocrit tubes were placed in radial grooves opposite each other with sealed ends away from the centre of the centrifuge. The centrifuge cover was screwed and lid closed. The tubes were spun at 11,00rpm for 10mins. The PCV were determined using a microhaemotocrit reader.

Red Blood Cell Count

4ml of diluted isotonic fluid was dispersed into a tube and mixture with 20 microtlitre blood sample. Counting was carried out using Neubauer counting chamber, 3mins after charging it.

White Blood Cell Count

0.4ml of white cell diluting fluid was dispersed into a small tube. 20microlitre of blood sample was measured and mixed with the diluents. The mixture was allowed to stand for 3mins for complete lyses of the red cells. Using a capillary tube,

some diluted blood was pipetted from the tube to fill the counting chamber. The cells were counted after they had settled for 1-2mins in the counting chamber.

3.13 Spectral Methods

3.13.1 GC-MS Method

Gas chromatography –mass spectroscopy (GC-MS) as the name implies is actually two techniques that are combined to form a single method of analyzing mixtures of chemicals. Gas chromatography separates the components of a mixture and mass-spectroscopy characterizes each of the components individually. By combining the two techniques, both qualitative evaluation of an extract containing a number of compounds can be achieved.

The GC component has a long, thin column containing a thin interior coating of a solid stationary phase (5% phenyl, 95% dimethylsiloxane polymer). This 0.25mm diameter column is called a capillary column. It is used for semi volatile, non-polar organic compounds. The capillary column is held in an oven programmed to increase the temperature gradually (or in GC terms, ramped). As the temperature increases, those compounds that have low boiling point elute from the column sooner than those that have higher boiling points. Therefore, there are actually two distinct separating forces, temperature and stationary phase interactions. As the compounds are separated, they elute from the column and enter a detector which creates an electric signal whenever the presence of a compound is detected. The greater the concentration in the sample, the bigger the signal. The signal is then processed by a computer, which then generate a graph from the signal (chromatogram).

The individual compounds eluted from the GC column enter the electron ionization (mass spectrometer) detector. There, they are bombarded with a stream of electrons causing them to break apart into fragments. A group of four electromagnets called a quadropole focuses each g\fragment though a slit and into the detector. The quadropoles are programmed by the computer and each quadropole cycle is referred to as scan. The computer records a graph for each scan.

The GC-MS has a library of spectra that can be used to identify an unknown chemical in the sample mixture. The library compares the mass spectrum in the library. It then reports a list of likely identifications along with the statistical probability of the match.

3.13.2 IR Method

The infra-red is a technique by which chemical bonds in different environments absorb varying intensities and at varying frequencies. Thus, IR spectroscopy involves collecting absorption information and analyzing it in the form of a spectrum. The frequencies at which there are absorptions of IR radiation (peaks as signals) can be correlated directly to bonds within the compound. The technique is useful for identifying certain functional groups in samples and can serve as a fingerprint for the extract constituents.

The Fourier Transform IR spectrometer uses a source of infra-red radiation such as nichrome wire or cooled rod of silicon carbide to produce a range of frequencies which are then separated into individual frequencies using a monochromator diffraction grating. The beam produced is then split into two and

one asses through the sample whilst to other is used as a reference beam. The two beams then converge on the detector which measures the difference in intensity and

then sends a proportional signal to the recorder. The resulting plot is a measure of transmission against frequency.

3.14 Anti-bacterial activity (Oyeleke and Manga, 2008)

Media preparation

28.0g of Nutrient Agar was dissolved in a liter of distilled water and sterilized at 121°C for 5mins using autoclave. After sterilization, it was allowed to cool to 45°C before dispensing on sterile Petri dishes and finally allowed to solidify before use. The test organism was inoculated into each of sterile nutrient broth in test tube and incubated at 37°C for 3hrs.

Sensitivity Test

The cork burrowing method was employed for the test. The cork borer of size 7mm was sterilized in an oven at 160°C for 1hr. It was then used to bore a hole in the nutrient agar plates. The essence is to create an environment to dispense the extracts. The dispensing of fractions(1.0mg/ml) was done after inoculation of test bacterial was effected. The plates were then incubated at 37°C for 2hrs. After incubation, the zones of inhibition were measured. The organisms selected for the test were *Salmonella typhi,Klebsiella pneumonea* and *Staphylococcus aureus*.

CHAPTER FOUR

RESULTS

4.0: Results

4.1: Analyses of the Crude Plant Extracts

The percentage aqueous crude extracts yield of the six plants analyzed are given in Table 4.1. The values ranged from 20% to 80%. The lowest yield (20%) was obtained from *Zizzipus spinachristi* while the highest (80%) was obtained from the stem bark of the *Anacardium occidentale*.

4.2: Safe Doses and Clinical Observations at Higher Doses

The safe doses and clinical observations at higher doses are as shown in Table 4.2. The aqueous extract of *A.occidentale* leaf had the lowest safe dose (300mg/kg bwt) while the aqueous extract of *Zizzypus spinachristi* leaves had the highest safe dose (900mg/kg bwt). At safe doses, the rats showed no apparent clinical adverse side effects when orally administered. However, at higher doses, similar clinical manifestations were observed for all the extracts.

Table 4.1: Percentage Aqueous Crude Extract Yield of the Plants used.

Plant Extracts	Crude extract yield %
Zizzypus spinachristi	20
Artemisia herba-alba	60
Terminalia glaucesceus	55
Moringa oleifera	50
Blighia sapida	40
Anacardium occidentale	70 80*

The crude extract was obtained from the leaves of the plants.

* % extract for the stem.

Table 4.2: Safe Dose and Clinical Observations of Aqueous Leaf Extracts of the Plants in rats.

Plant (Aqueous extract leaf)	Safe doses (mg/kg b.wt p.o)	Observation at higher doses (mg/kg bwt)
Zizzypus spinachristi	900	At>1000 weakness, drowsiness, mortality
Artemisia herba-alba	525	At>650 Weakness, intense drowsiness, mortality
Terminalla glaucesceus	550	At>600 Weakness, drowsiness, mortality
Moringa oleifera	500	At>600 Weakness, salivation, mortality
Blighia sapida	400	At>500 Weakness, diarrhea, mortality
Anacardium occidentale	300	At>300 Weakness, intense drowsiness, mortality

BW = Body Weight

P.O = Per-Oral

4.3: The Lethal Doses of the Extracts

The lethal doses (LD50) determined for all the plants are as expressed in Table 4.3. The aqueous extract of *Anacardium occidentale* leaves had the lowest LD50 at 450mg/kg.bwt while the aqueous extract of *Zizzypus spinachristi* leaves had the highest LD50 at 1,250mg/kg.bwt. The lethal doses of the aqueous extracts of the other four plants are as indicated in the table.

4.4: The Percentage Glucose Reduction of Different Plant Aqueous Extract

The results of the glucose reduction ability of different plant extracts tested on alloxan induced rats are indicated in Table 4.4. The aqueous extract of *Anacardium occidentale* and *Moringa oleifera* leaves had the highest percentage glucose reduction of 74.2% and 74.0% respectively, while *Artemisia herba-alba* had the lowest percentage glucose reduction (43.4).

Table 4.3: Lethal Dose (LD 50) of Aqueous Leaf Extracts of the Plants in Rats.

Plant (Aqueous Extracts)	LD50 (mg/kg b.wt)
Zizzypus spinachristi	1,250
Artemisia herba-alba	750
Terminalia glaucesceus	650
Moringa oleifera	700
Blighia sapida	600
Anacardium occidentale	450

Table 4.4: Percentage Glucose Reduction of Aqueous Leaf extracts of the Plants in Alloxan induced rats.

Plant (Aqueous Extract)	% glucose reduction (Alloxan-induced models)
Zizzypus spinachristi	43.7
Artemisia herba-alba	43.4
Terminalia glaucesceus	44.3
Moringa oleifera	74.0
Blighia sapida	51.2
Anacardium occidentale	74.2

4.5: Percentage Glucose Reduction of Stepwise Fractions of *A. occidentale.*

The results of the percentage glucose reduction by fractions from stepwise fractionation of the *Anacardium occidentale* leaves and stem in Alloxan induced rats were as presented in Table 4.5. The ethanolic fraction of *Anacardium* leaves (200mg/kgb. wt) had the highest percentage glucose reduction (46.2) while the hexane fraction at same dose had the lowest percentage glucose reduction (24.0). At higher dose (300mg/kg b.wt), lower response was obtained for the two fractions. The ethylacetate fraction of *Anacardium* stem bark at both 200mg and 300mg had the highest percentage glucose reduction (4.2 and 4.4) while hexane fraction had the least (1.3). All the fractions from the stepwise fractionation of the stem exhibit some mild activities (Table 4.5).

Table 4.5: Percentage Glucose Reduction of stepwise fractions of *A. occidentale* in Alloxan induced rats.

Stepwise Fractions	Anacardium Leaf		Anacardium Stem Bark	
	% Glucose Reduction (200mg/kg bwt)	% Glucose Reduction (300mg/kg bwt)	%Glucose Reduction (200mg/kg bwt)	% Glucose Reduction (300mg/kg bwt)
Hexane	24.0	0.6	1.3	1.3
Ethylacetate	45.8	20.0	4.2	4.4
Ethylacetate / Ethanol	27.0	20.0	3.2	4.1
Ethanol	46.2	20.0	1.2	4.1

4.6: Antibacterial Activity of Stepwise Fractions of Leaf Extract of *A. occidentale*.

The results of antibacterial activity of the stepwise fractions of Anacardium occidentale leaf are expressed in Table 4.6. The ethylacetate/ethanol (1:1) and ethanol fractions inhibit *Klebsiella pneumonae* species with zones of inhibition of 13mm and 11mm respectively. The ethylacetate fraction inhibits *Staphylococcus aureus* with zone of inhibition of 12mm while hexane fraction inhibits *Salmonella typhi* with zone of inhibition of 10mm.

4.7: The Phytochemicals in Different Plant Extracts

The results of phytochemicals detected in different plant extracts are presented in Table 4.7. All the six plants showed presence of some phytochemicals.

Table 4.6: Antibacterial Activity of Stepwise fractions of *Anacardium occidentale* leaf

Stepwise Fractions	Zones of Inhibitions (mm)		
	Kliebsella P.	*Salmonella Typhi*	*Staphylococcus aureus*
Hexane	0	10mm	0
Ethylacetate	0	0	12mm
Ethylacetate / ethanol	13mm	0	0
Ethanol	11mm	0	0

Table 4.7: Phytochemicals detected in the different plant extracts

Plants	Alkaloid	Anthraquinones	Tannias	Saponins	Phlobatannins	Resins	Vol. oils	Flavonoids	Terpenes	Cardiarc glycosides	Indole alkaloids	Cyanophoric Glycosides	Balsams
Zizzypus spinachristi	-	++	+++	++	-	+	+	+++	+++	-	-	-	-
Artemisia herba-alba	++	++	++	++	-	-	-	++	-	-	+	+	-
Terminalia glaucescens	++	++	++	-	-	-	+	++	-	-	++	-	-
Moringa oleifera	+++	++	+++	++	-	-	-	+++	-	-	+	+	-
Blighia sapida	++				-	-	-	+	+	+	+	-	-
Anacardium occidentale	++	++	++	++	+	-	+	+	++	+	+	+	-

Keys: +++ = Highly positive - = Negative.
++ = Moderately positive
+ = Faintly positive

4.8: Average Daily Food Intake of Rats Administered Crude Ethanolic Extract of *A. occidentale* Leaf.

The average daily food intake of rats administered crude ethanolic extract of *Anacardium occidentale* for acute toxicity tests were indicated in Table 4.8.The results indicates decrease in average daily food intake for all the experimental groups. At the end of the 7 days period of the experiment, the weight of the rats in group I decreased from 22.42g/day to 14.93g/day while in group II rats, the weight decreased from 21.05g/day to 10.45 g/day.

4.9: Biochemical Parameters of Rats Administered ethanolic Extract of *A. occidentale* Leaf

Table 4.9 showed biochemical parameters namely ALT, AST, total protein, creatinine and urea of rats administered ethanolic extract of *A.occidentale* leaf. The ALT and AST activity decreased significantly ($P<0.05$) for all the groups at the end of 6weeks study period. The total protein decreased significantly ($P<0.05$) at the end of 6weeks study period for all the groups. The creatinine and urea levels increased significantly ($p<0.05$) for all the groups when compared with the control.

Table 4.8: Average Daily Food Intake of Rats Administered crude ethanolic extract of *A.occidentale* Leaf (g/rat)

	Days of Treatment		
	0	4	7
Control	20.53	23.33	25.52
Group I	22.42	16.43	14.93
Group II	21.05	11.36	10.45

Group I (rats fed 2000mg)

Group II (rats fed 4000mg)

Table 4.9: Biochemical Parameters of Rats Administered Ethanolic Extract of *A. occidentale* leaf

Parameters	Control		Group I		Group II		Group III	
	0	42	0	42	0	42	0	42
ALT (U/L)	49.94±0.2	49.73±0.3	50.12±0.5	48.27±0.2*	49.93±0.8	46.39±0.2**	50.49±0.4	41.88±0.9+
AST (U/L)	52.8±0.4	53.4±0.1	53.0±1.2	51.7±0.5*	52.5±2.0	48.2±0.6**	52.6±1.6	44.5±0.6+
Total protein (g/dl)	6.76±0.01	6.74±0.04	6.84±0.04	6.29±0.04	6.79±0.207	6.36±0.05	6.82±0.05	6.39±0.09
Creatinine (mmol/l)	10.3±0.1	14.5±0.2	15.3±0.21	17.2±0.12*	15.1±0.31	16.8±0.118**	18.5±0.21	19.1±0.22+
Urea (mmol/l)	32.1±0.21	35.3±0.11	30.4±0.2	34.3±0.41*	36.2±0.21	37.2±0.2**	39.1±0.22	40.1±0.11+

The Values are expressed as mean ± SEM

Group I (rats fed2000mg) Vs Control *P<0.05

Group II (rats fed 4000mg) Vs Control **P<0.05

Group III (rats fed 6000mg) Vs Control +P<0.05

4.10: Percentage Glucose Reduction of Column Effluents of *A. occidentale* leaf.

Table 4.10 showed the percentage glucose reduction of the column effluents of *Anacardium* at varying doses. Ethylacetate fraction at both 200mg and 300mg showed high percentage glucose reduction (54.1 and 59.6) when compared to the oral hypoglycemic drug-metformin (500mg/kg b. wt). The ethylacetate fraction at 300mg/kg b.wt had a higher activity than the metformin drug.

Table 4.10: Comparative Hypoglycemic Effects of **Metformin and Column Effluents of Ethanolic Fraction of *Anacardium occidentale* Leaf extract

Effluents/Drug	% glucose reduction
Ethylacetate Effluent (300mg)	59.6
Ethylacetate Effluent (200mg)	54.1
Ethylac: Ethanol Effluent (300mg)	53.5
Ethylac: Ethanol Effluent (200mg)	50.2
Ethanol Effluent (300mg)	0.72
Ethanol Effluent (200mg)	0.33
Metformin (500mg)	54.1

* Effective Effluents Compared with Metformin Drug.

** Metformin – Oral Hypoglycemic drug.

4.11 Effect of Ethanolic Extract of *A. occidentale* Leaf on the Body weight of rats.

The effect of ethanolic extract of *A. occidentale* on the body weights of rats are as shown in Fig4.1. The body weight of Group I rats increased from day0 to day21 and decreased significantly ($P<0.05$) for the remaining period of study. The body weight for Group II also increased from day0 to day7 and then decreased significantly ($P<0.05$) for the remaining study period. For Group III rats, there was significant ($P<0.05$) decrease in body weight throughout the period of study.

4.12 Effect of Ethanolic Extract of *A. occidentale* Leaf on Packed Cell Volume.

The results of the effect of ethanolic extract of *A. occidentale* leaf on PCV are presented in Fig 4.2. The PCV values obtained for all the groups fluctuated throughout the period of study. However, there was no significant ($P>0.05$) difference between the control and the groups at the end of the experiment.

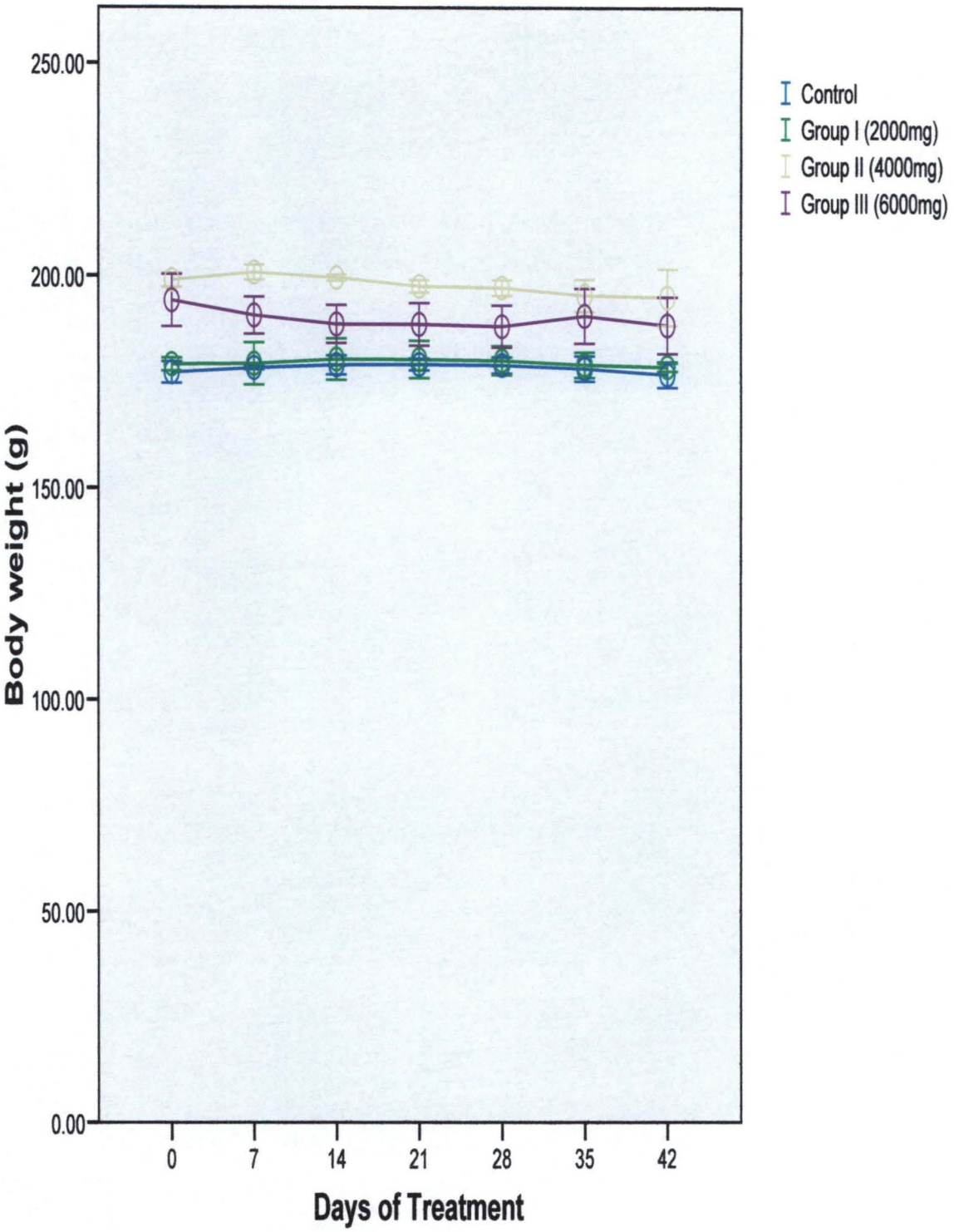


Fig4. 1: Effect of ethanolic extract of *A. occidentale* leaf on body weight of rats.

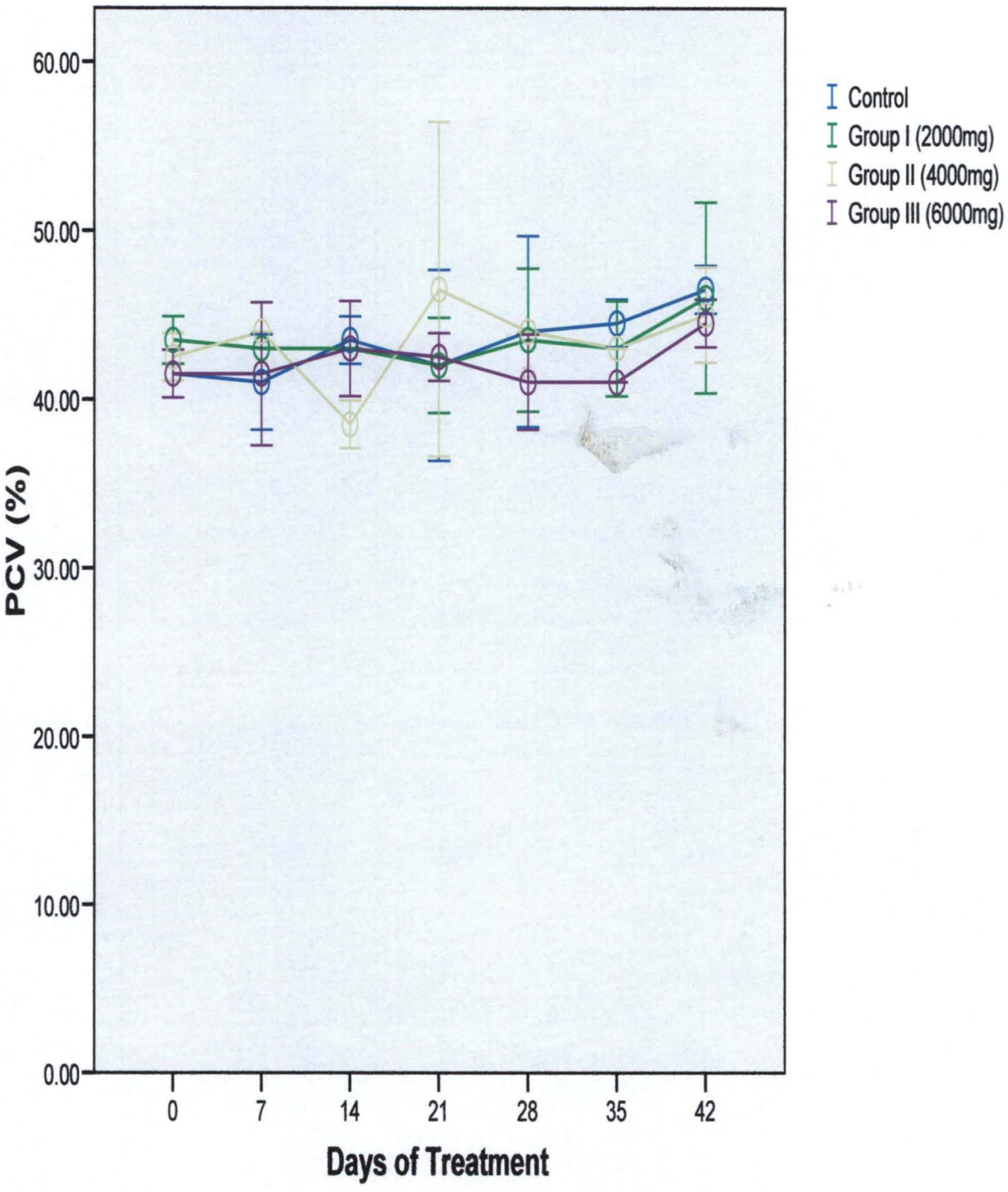


Fig 4.2: Mean percentage PCV values of rats fed with ethanolic extract of *A. occidentale leaf*

4.13 Effect of Ethanolic Extract of *A. occidentale* Leaf on Red Blood Cell Count.

The results of the effect ethanolic extract of *A. occidentale* on RBCs are as indicated in Fig 4.3. The RBC values for all the groups fluctuated throughout the period of study (0-42 days). However, there was no significant ($P>0.05$) difference between the control and the groups.

4.14 Effect of Ethanolic Extract of *A. occidentale* Leaf on White Blood Cells Count.

The results of the effect of ethanolic extract of *A. occidentale* leaf on WBCs of rats are shown in Fig 4.4. There was initial increase in WBC values for all the groups (0-7 days) but the values fluctuated for the remaining period of study. However, there was no significant difference ($P>0.05$) between the control and the groups.

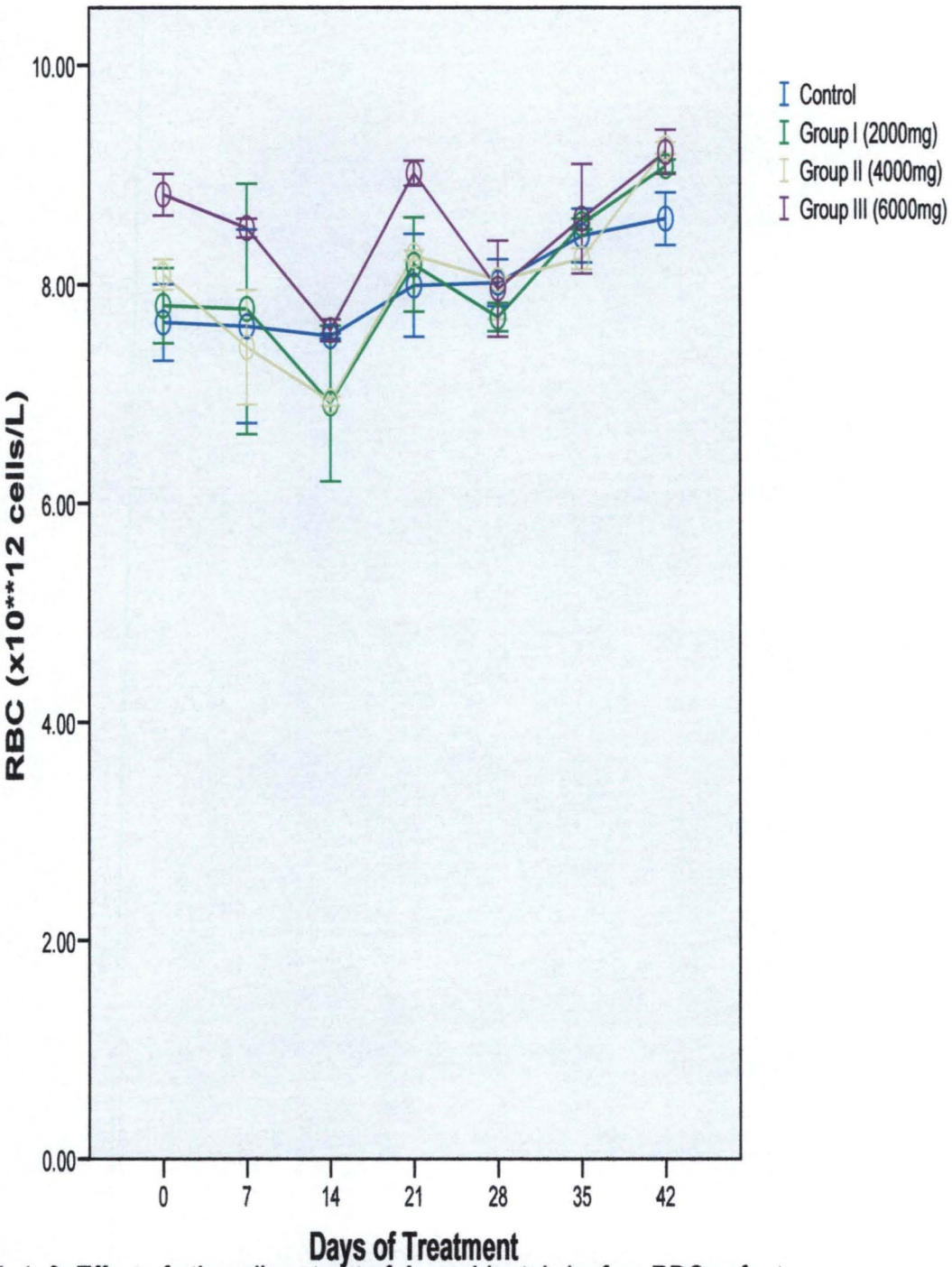


Fig4. 3: Effect of ethanolic extract of *A. occidentale* leaf on RBCs of rats.

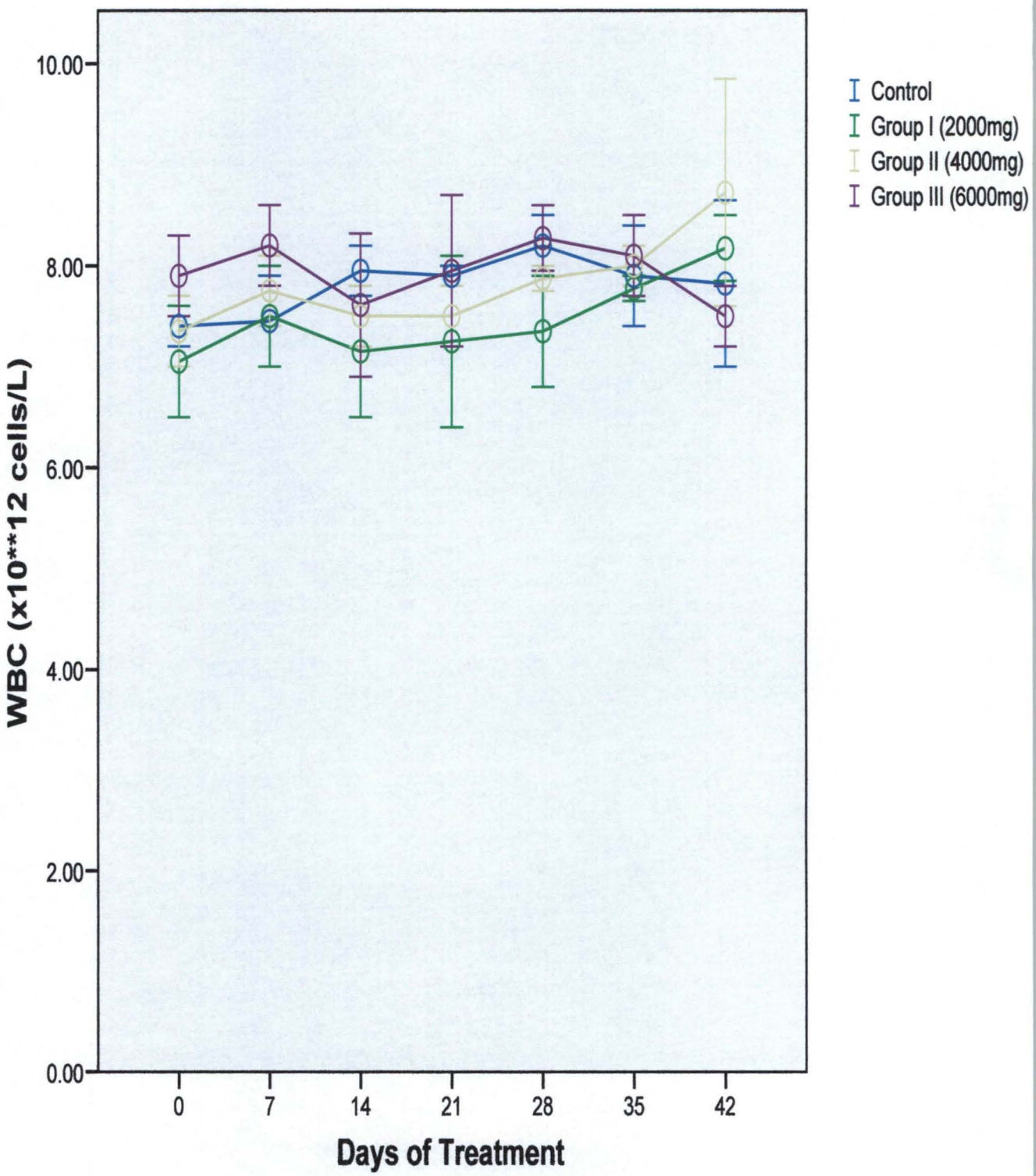


Fig 4.4: Effect of ethanolic extract of *A. occidentale* leaf on WBCs of rats.

4.15 Effect of Aqueous Extract of *Blighia sapida* Leaf on body weight and some serum biochemical parameters of Normoglycemic and Diabetic rats

The results of the effect of aqueous extract of *Blighia sapida* (400mg/kg bwt) leaf are presented in Figs 4.5-4.12. For normoglycemic rats, the administration of the extract resulted in loss of weight in the rats (Fig 4.5). However, the weight loss was not significant ($P>0.05$) compared with control. The extract significantly ($P<0.05$) lowered the serum glucose levels (82.00 ± 11.17 to 52.33 ± 10.15mg/dl) (Fig 4.6). The triglyceride values decreased from day 0 to day 7 but continued to increase for the remaining period of study. These changes were however not significant at $P>0.05$ (Fig 4.7). The total protein level increased from day 0 to day 14 and fluctuates for the remaining study period. The increase was however significant ($P >0.05$) compared to control (Fig 4.8). For diabetic rats, the extract significantly ($P<0.05$) increased the weight of the rats during the period of study as depicted in Fig 4.9. The extract lowered the glucose levels from $198.2\pm$ 12.10 to 97.7 $\pm$2.10mg/dl (Fig4.10). The decrease was however significant ($P<0.05$) compared with control. The triglyceride levels significantly ($P<0.05$) decreased while the protein levels increased significantly as shown in Figs 4.11 and 4.12 respectively. Generally, there was significant ($P <0.05$) difference between the normoglycemic and diabetic rats for serum glucose levels.

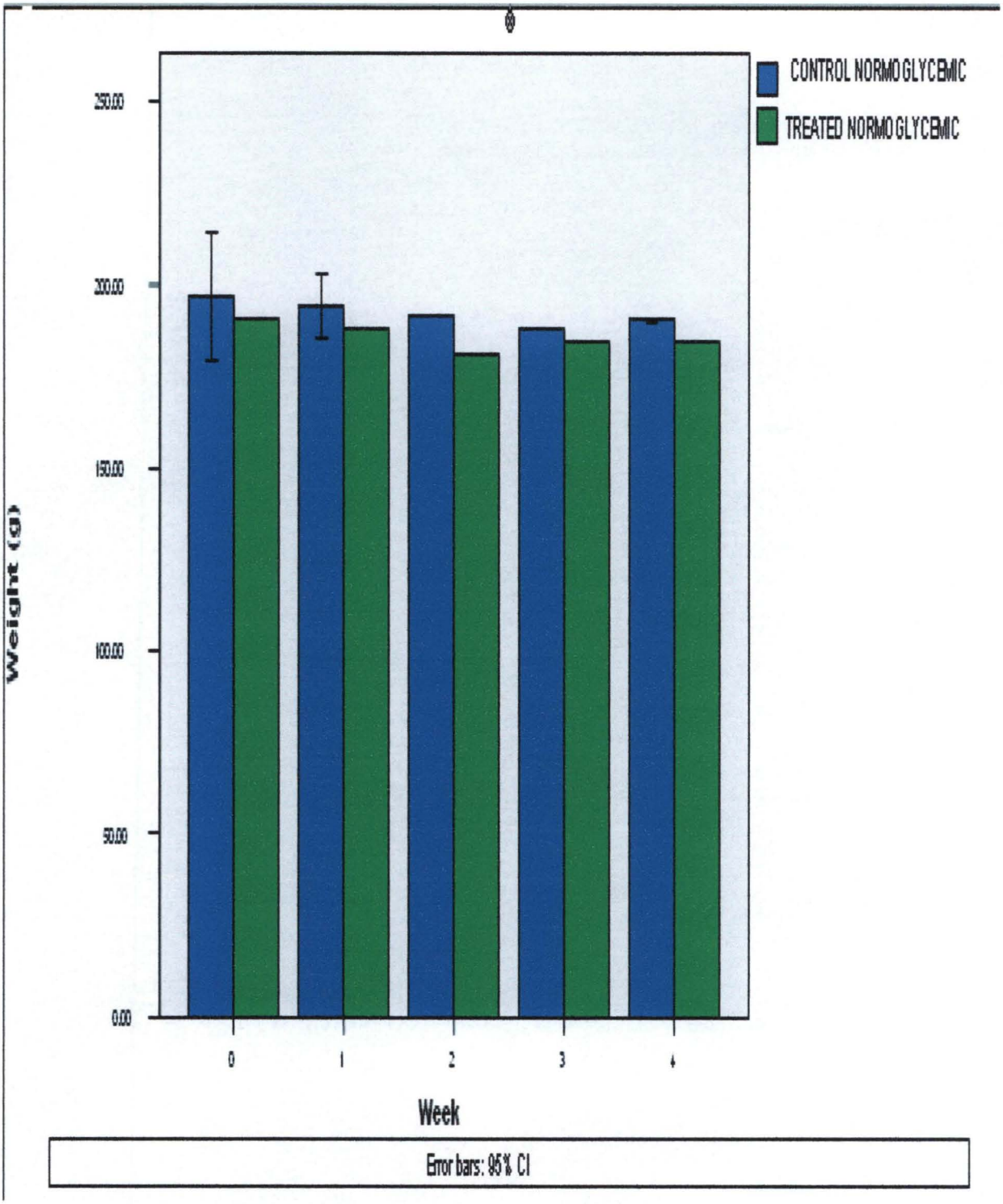


FIG 4.5:Effect of Aqueous extract (400mg/kg bwt) of *Blighia sapida* Leaf on weight of rats.

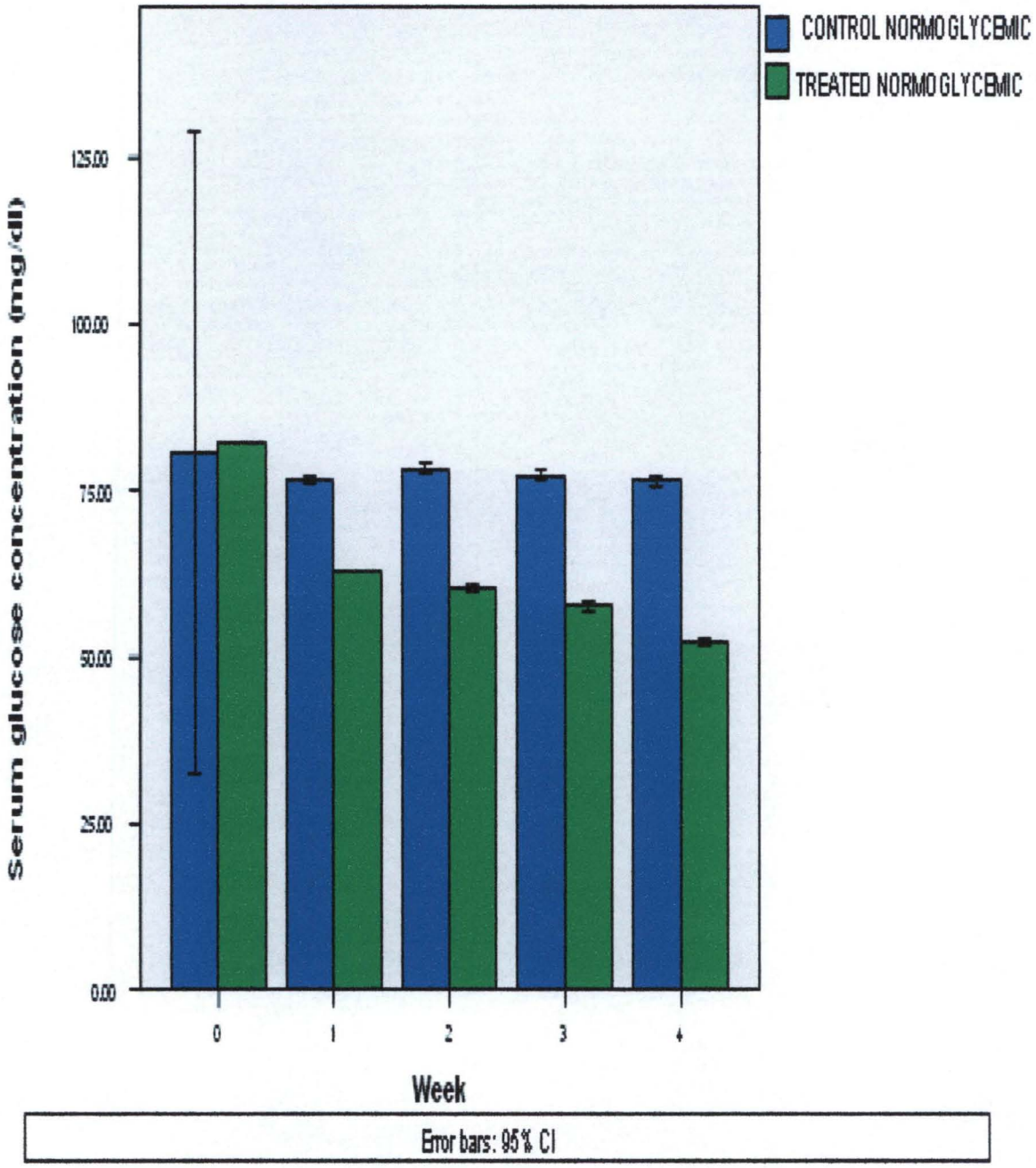


FIG 4.6: Effect of Aqueous extract (400mg/Kg b.wt) of *Blighia sapida* on serum glucose concentration in rats.

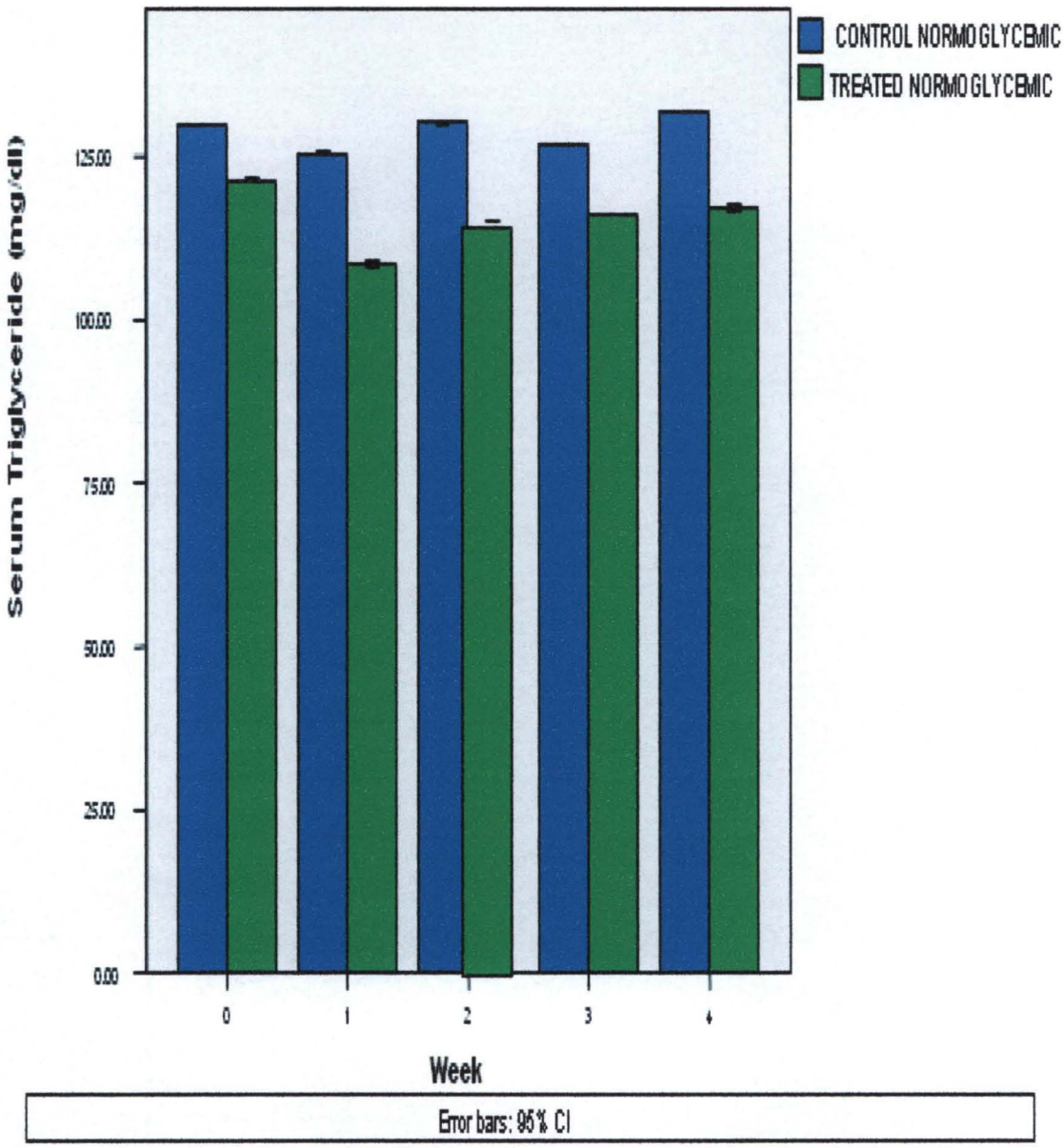


FIG 4.7: Effect of Aqueous extract (400mg/kg bwt) of *Blighia sapida* leaf extract on serum triglyceride in rats.

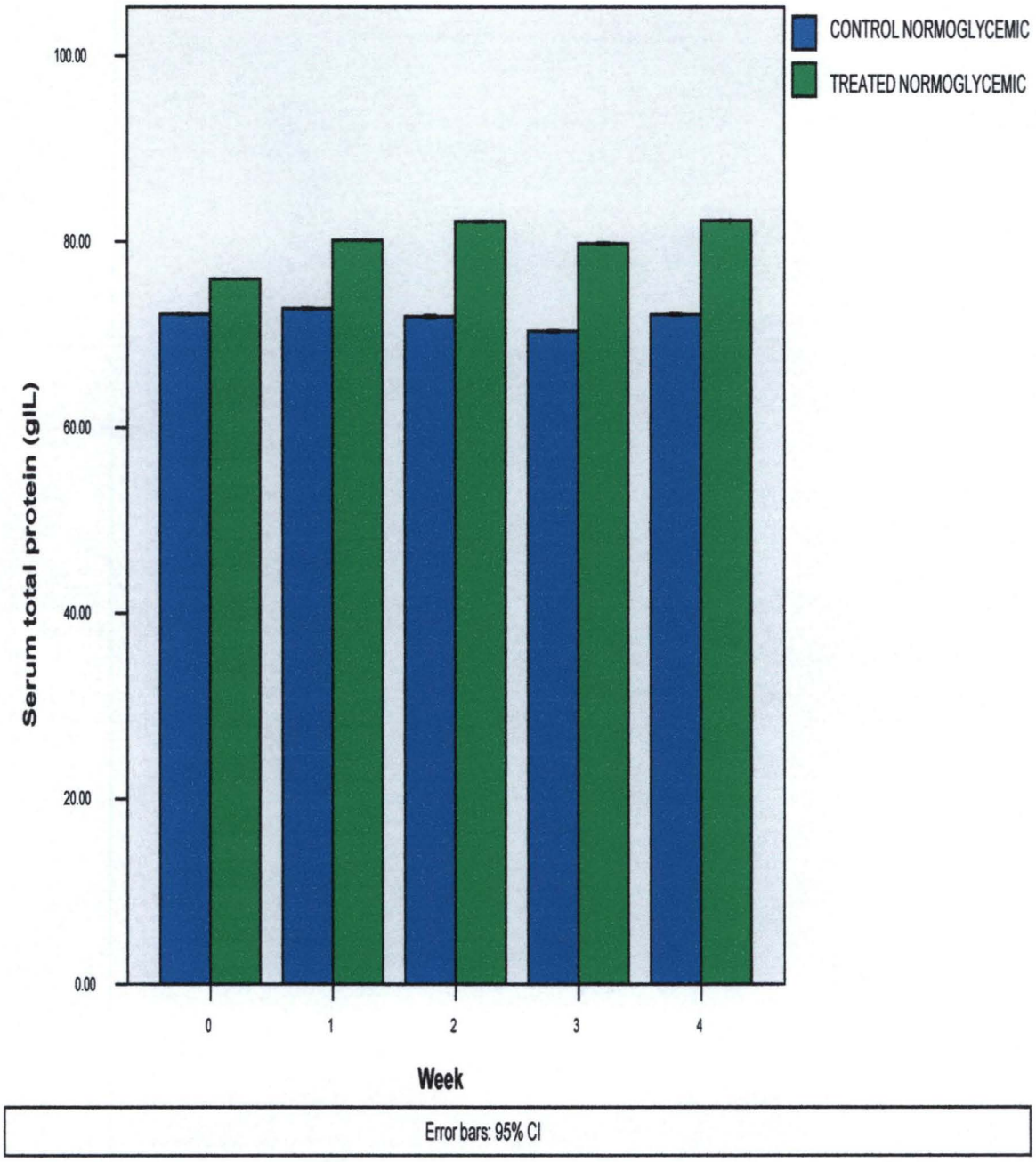


FIG 4.8: Effect of Aqueous extract (400mg/kg bwt) of *Blighia Sapida* Leaf on serum total protein in rats.

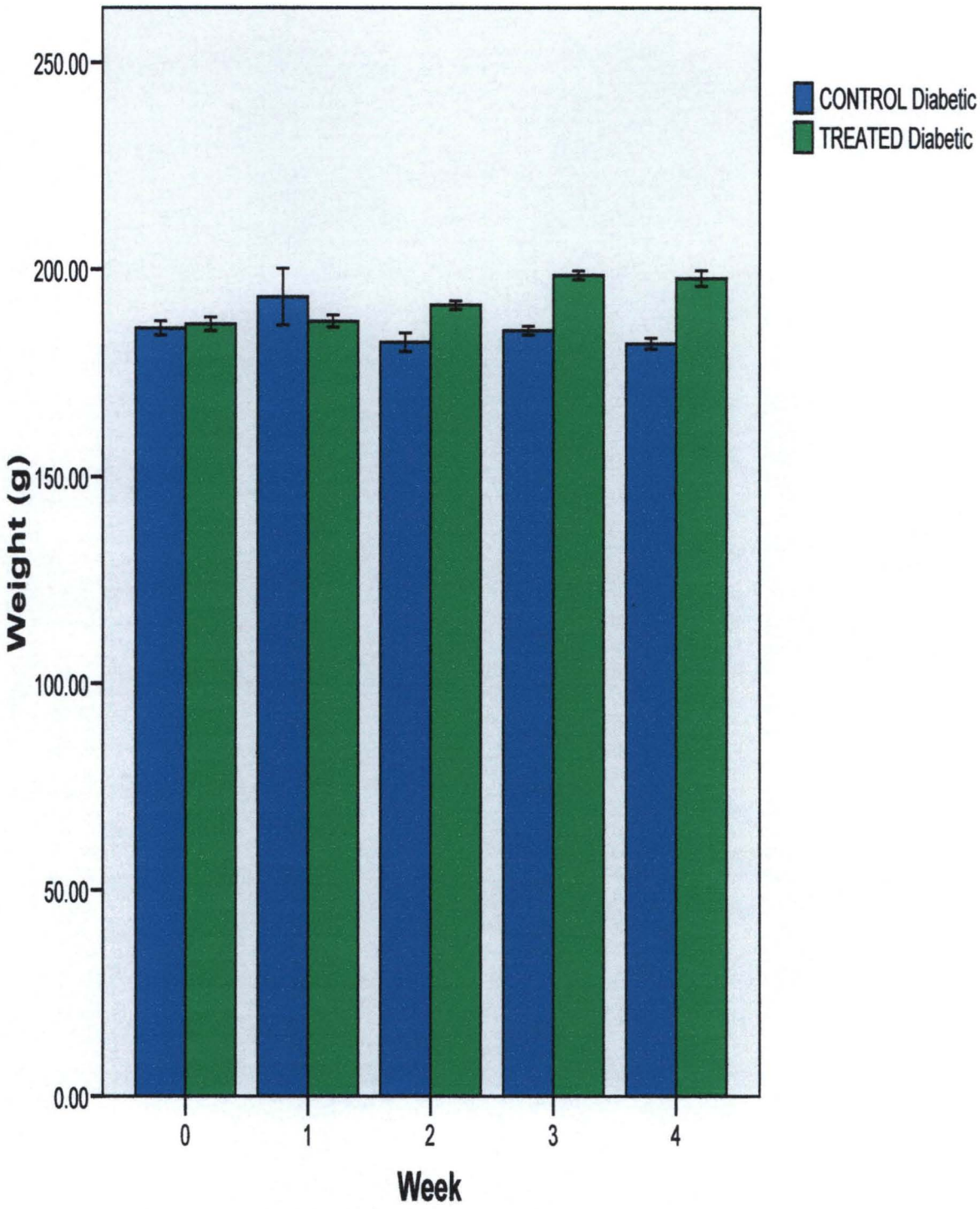


Fig4. 9: Effect of Aqueous extract (400mg/kg bwt) of *Blighia sapida* on weight of diabetic rats

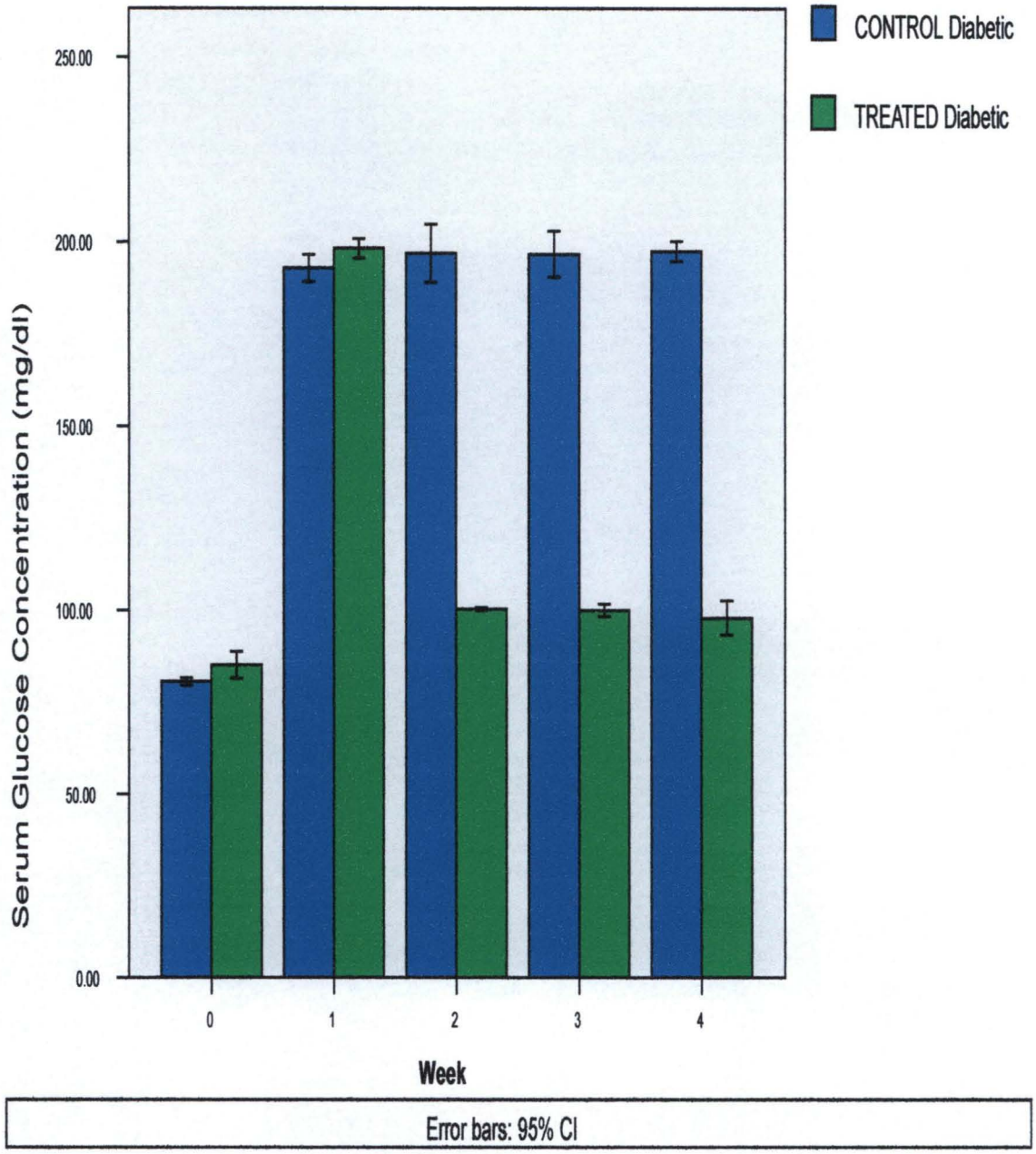


FIG 4.10: Effect of Aqeous extract(400mg/kg bwt) of *Blighia sapida* Leaf on serum glucose concentration in diabetic rats

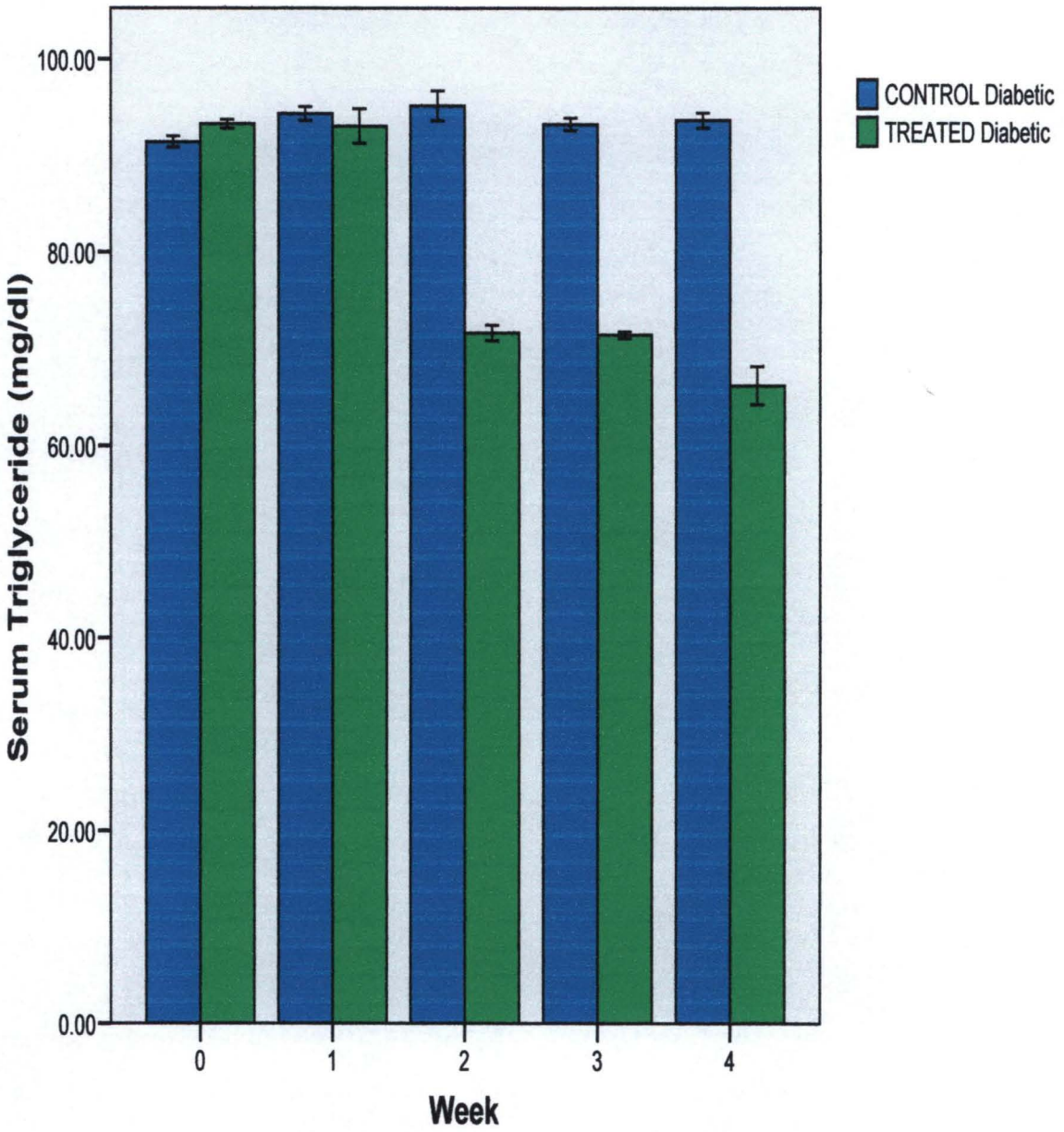


Fig 4.11: Effect of Aqueous extract (400mg/kg bwt) of *Blighia sapida* on serum Triglyceride levels in diabetic rats

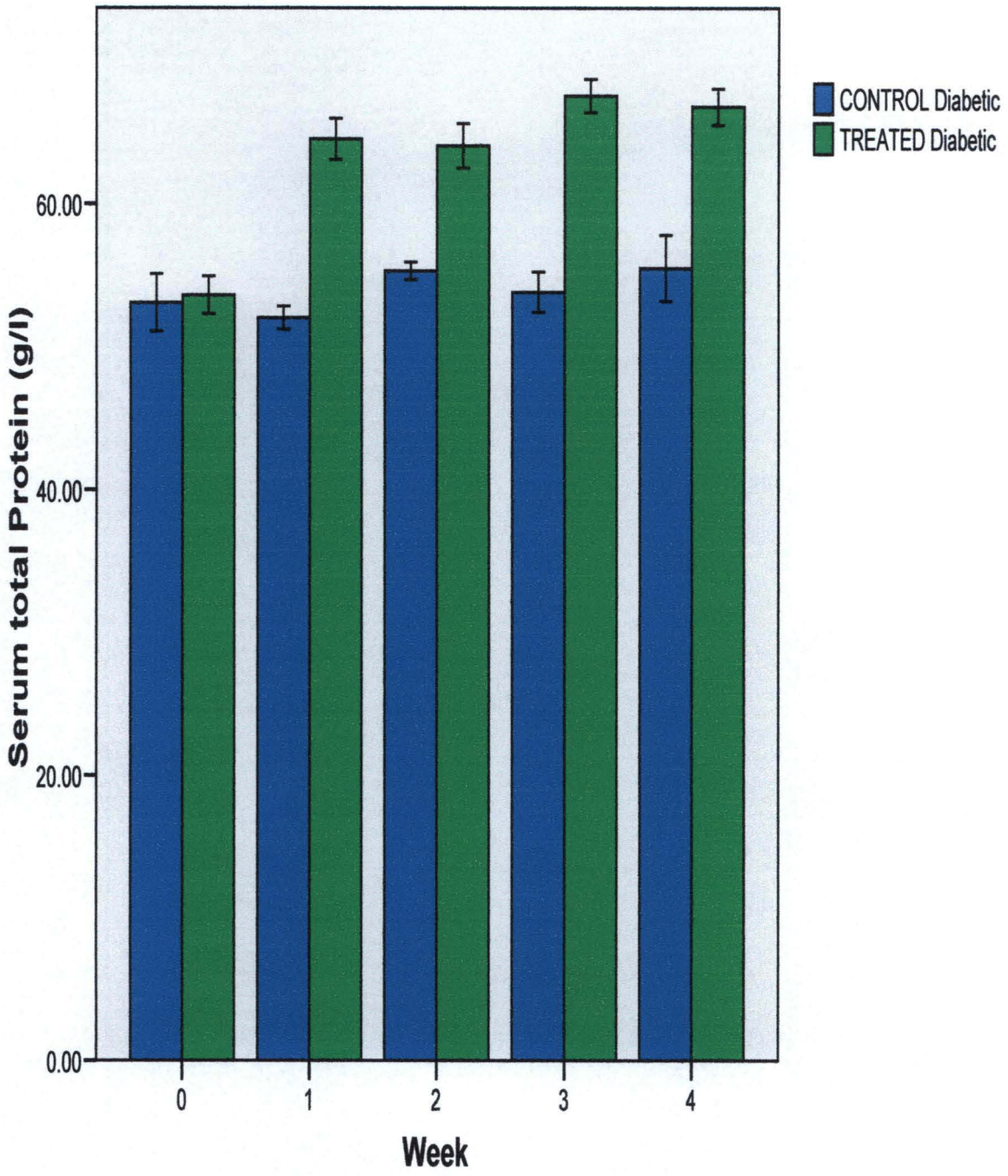


Fig 4.12: Effect of Aqueous extract (400mg/kg bwt) of *Blighia sapida* on serum total protein in diabetic rats.

4.16 Effect of Aqueous Extract of *Anacardium occidentale* Leaf on body weight and some serum biochemical parameters of Normoglycemic and Diabetic rats.

The results of the hypoglycemic properties of the aqueous extract of *Anacardium occidentale* (300mg/kg b.wt) are shown in Figs 4.13-4.20.For normoglycemic rats, the extract significantly ($P<0.05$) decreased the weight and serum glucose levels as presented in Figs 4.13 and 4.14 respectively. However the triglyceride and protein levels increased significantly for the study period as shown in Figs 4.15 and 4.16 respectively. For diabetic rats, the weight as a result of extract administration decreased the weight of the rats from 154.67 $\pm$ 10.2 to 147.10 $\pm$ 11.2g. The decrease was however not significant ($P>0.05$) for the period of the study (Fig 4.17).The extract significantly ($p<0.05$) lowered glucose levels from 210.80$\pm$12.2 to 54.47$\pm$2.01mg/dl for the period of the study compared to control (Fig 4.18).The triglyceride levels decreased from 63.3$\pm$4.10 to 48.1$\pm$2.01mg/dl. However, the decrease was significant ($P<0.05$) compared with

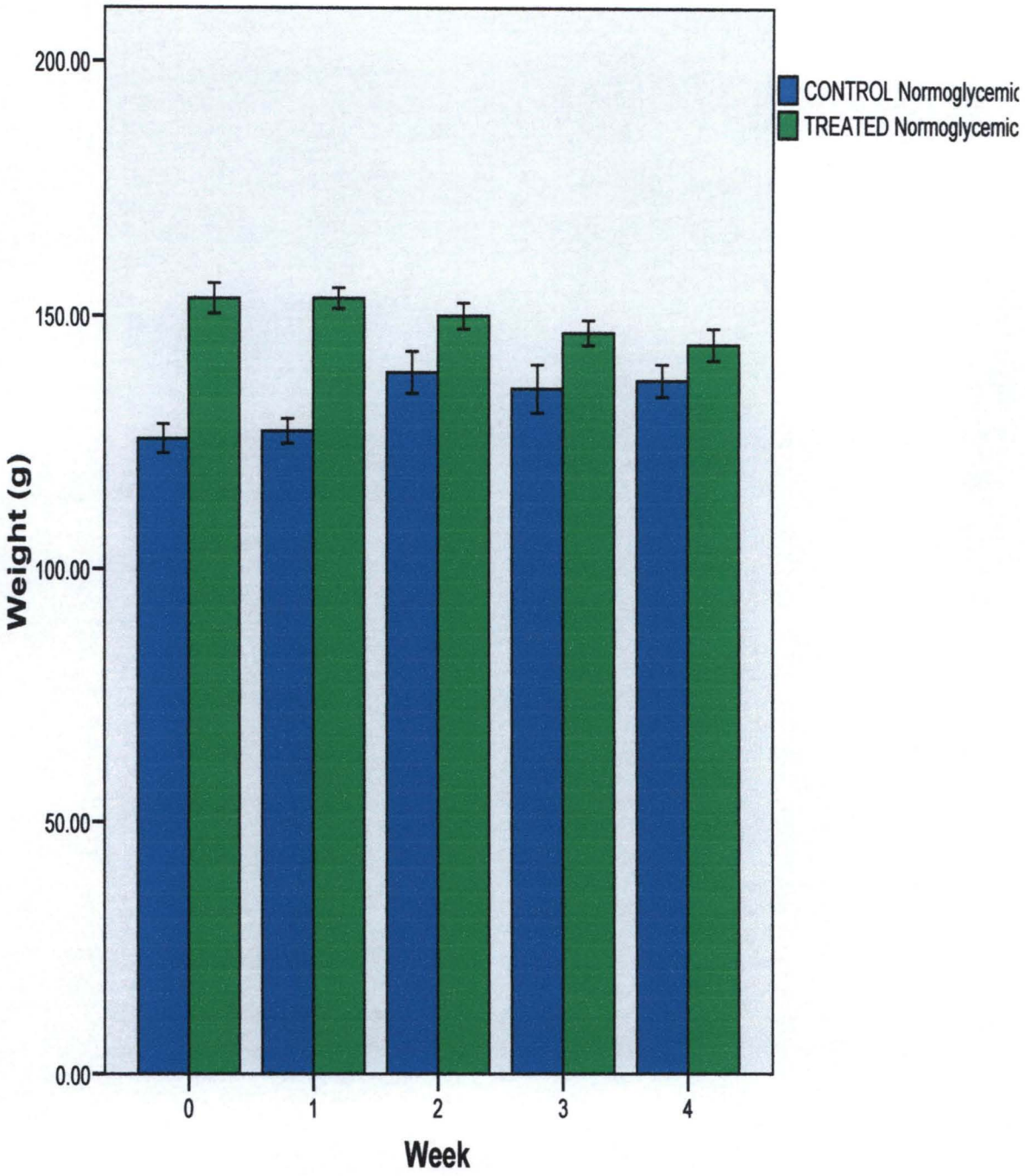


Fig 4.13: Effect of Aqueous extract (300mg/kg bwt) of *Ancardium occidentale leaf* on weight of rats

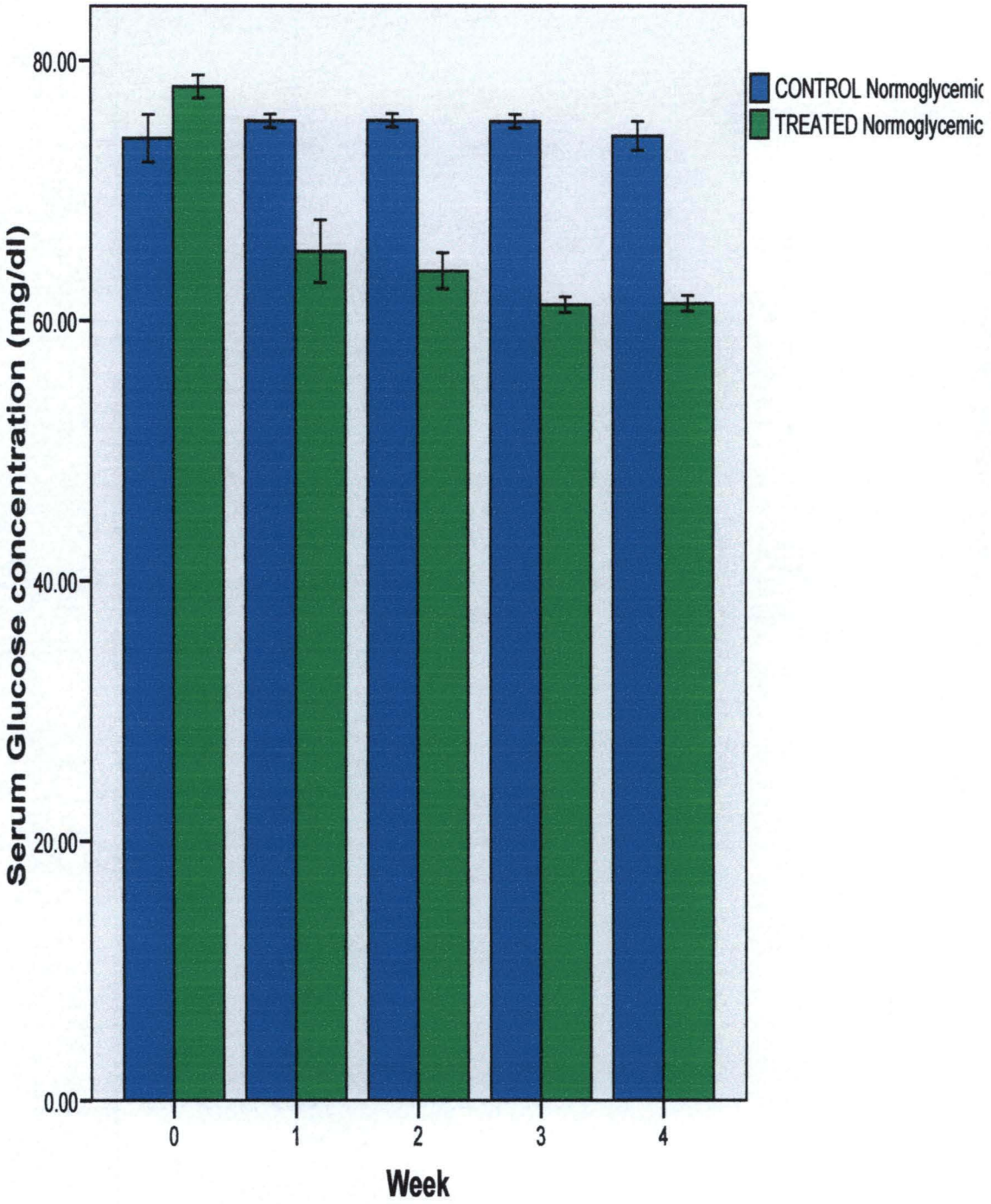


Fig 4.14: Effect of Aqueous extract (300mg/kg bwt) of *Ancardium occidentale* on serum glucose concentration in rats.

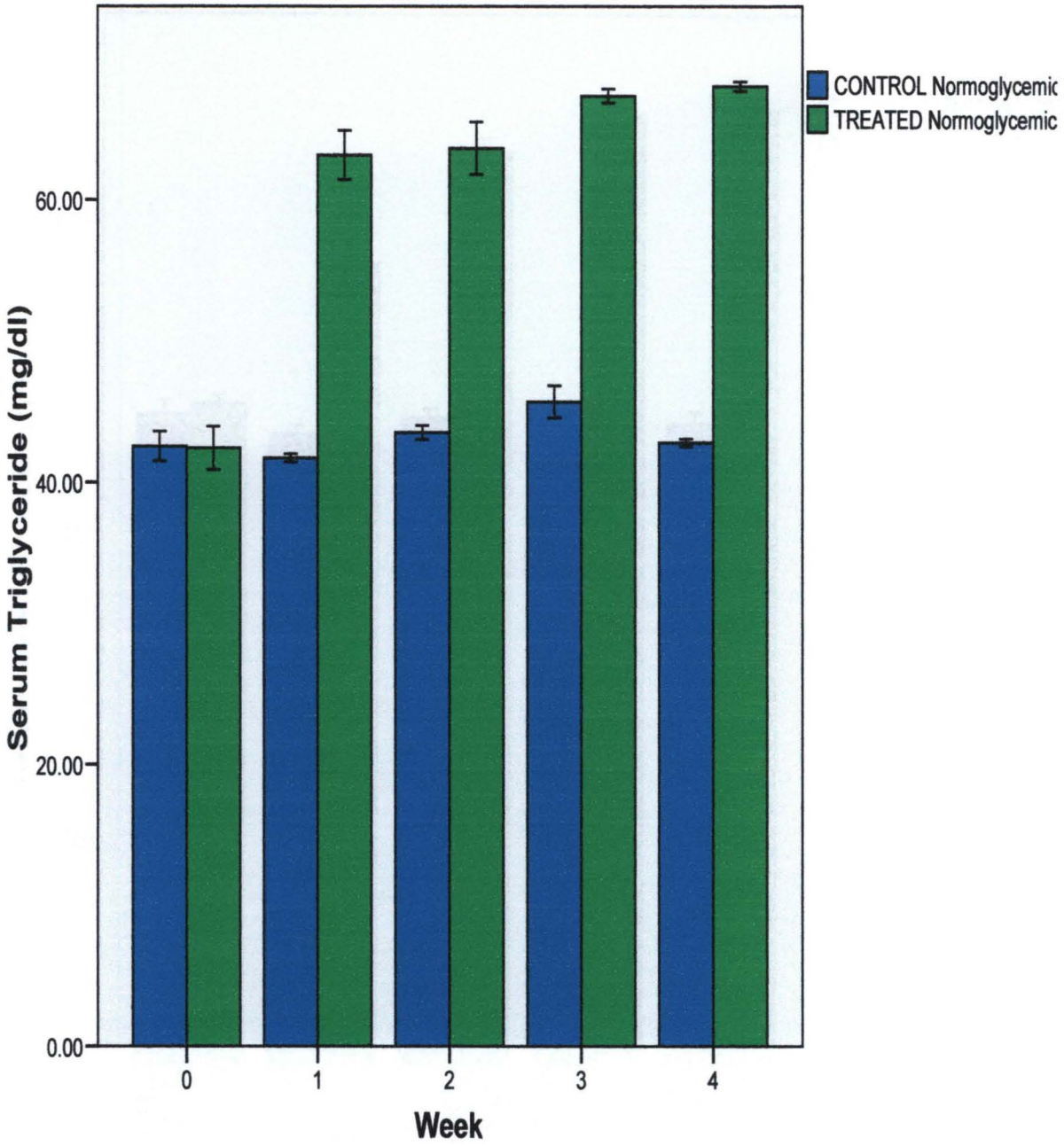


Fig 4.15: Effect of Aqueous extract (300mg/kg bwt) of *Anacardium occidentale leaf* on serum triglyceride levels in rats.

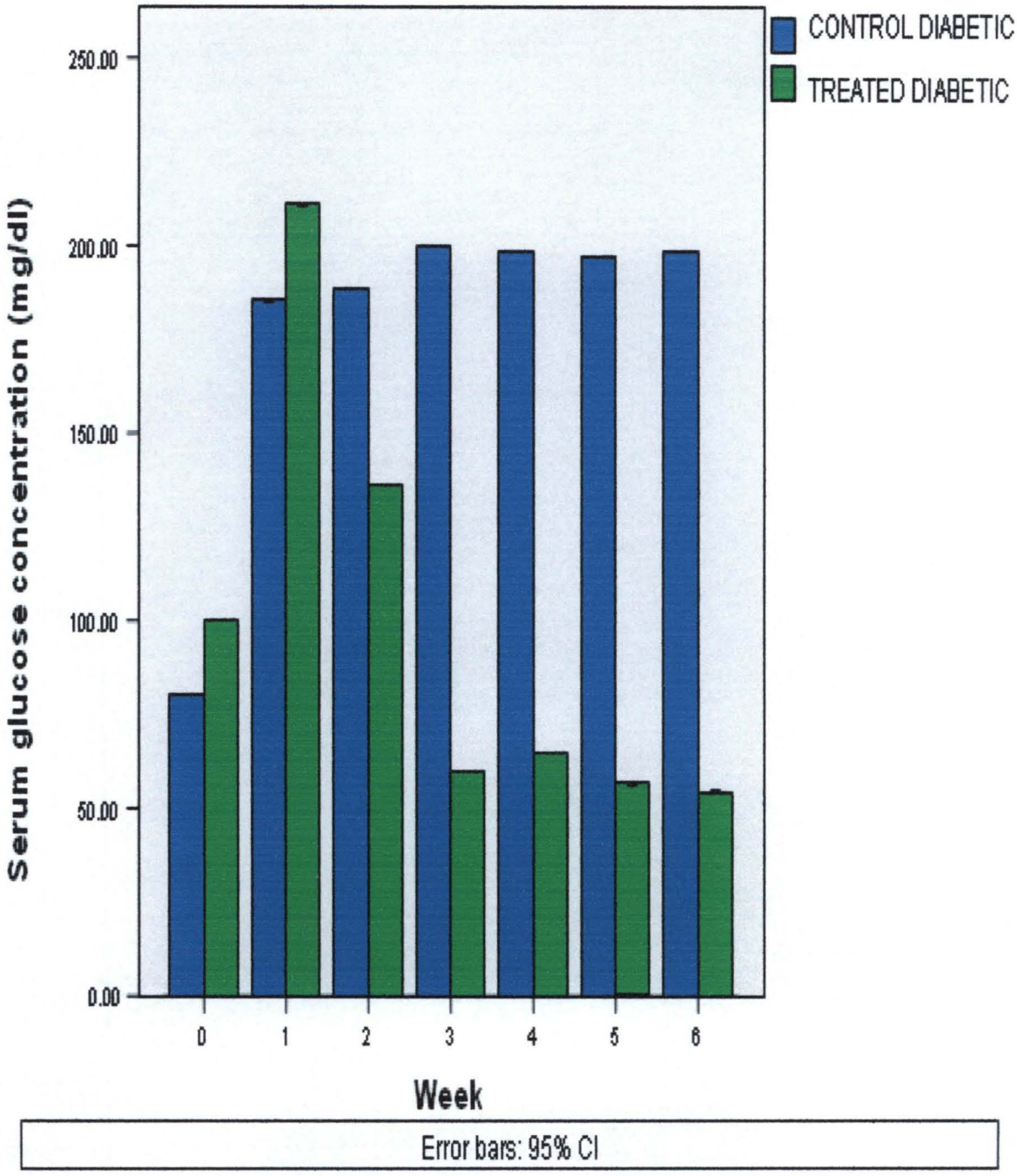


FIG 4.18. Effect of Aqueous extract (300 mg/Kg b.wt) of *Anacardium occidentale* Leaf on serum glucose concentration in diabetic rats.

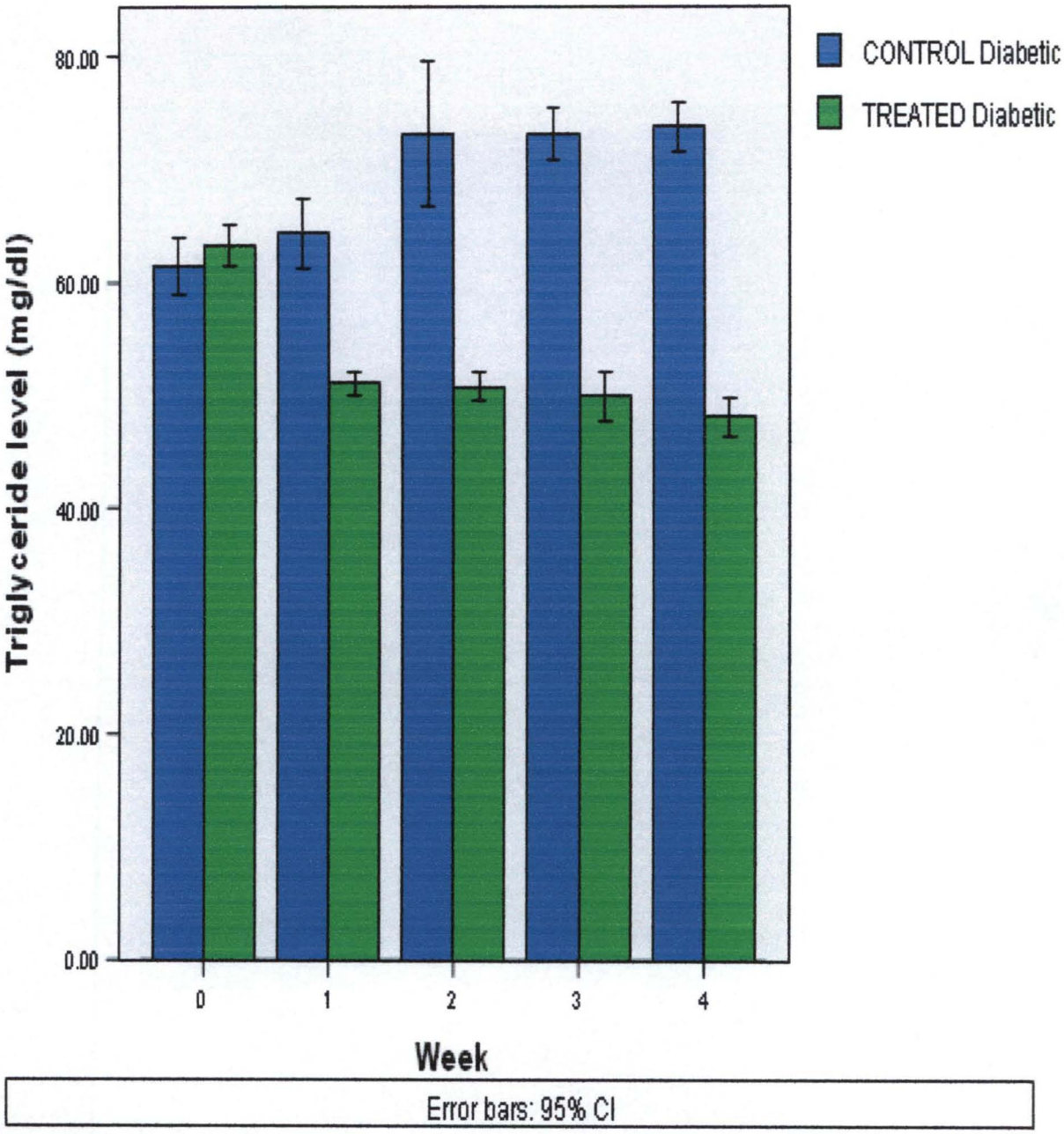


FIG 4.19: Effect of Aqueous extract (300mg/kg bwt) of *Anacardium occidentale* Leaf on serum Triglyceride levels in diabetic rats.

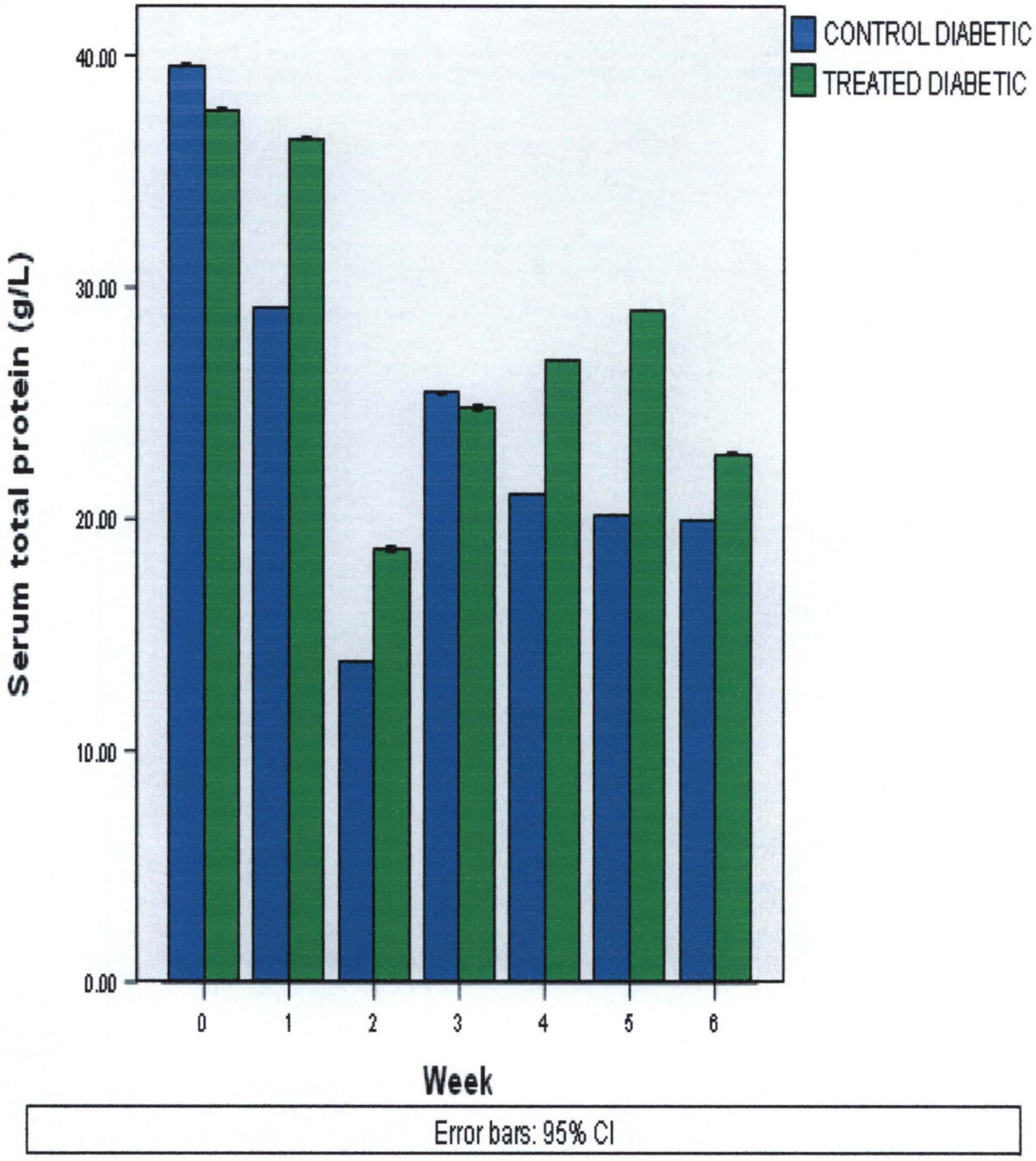


FIG 4.20: Effect of Aqueous extract (300mg/kg bwt) of *A. occidentale* Leaf on serum total protein in diabetic rats.

4.17 Effect of Aqueous Extract of *Moringa oleifera* Leaf on body weight and some serum biochemical parameters of Normoglycemic and Diabetic rats.

The results of the hypoglycemic properties of aqueous extract of *Moringa oleifera* (500mg/kg b.wt) are depicted in Figs 4.21-4.28. For normoglycemic rats, the extract administration results in weight decrease during the period of study. The decrease was however not significant ($P>0.05$) compared with control (Fig 4.21).The glucose level decreased non significantly ($P>0.05$) for the period of the study (Fig4.22) while the protein level increased significantly ($P<0.05$) for the period of the study as presented in Fig 4.23. The triglyceride level of the rats fluctuates during the period of study as presented in Fig 4.24. However, there was significant difference ($P<0.05$) between the normoglycemic and diabetic rats for serum glucose levels. For diabetic rats, the weight and triglyceride levels of the rats fluctuates for study period as shown in Figs 4.25 and 4.27 respectively.However, the extract significantly ($p<0.05$) lowered the glucose levels (209.8 ± 12.1 to 54.47 ± 2.01mg/dl) for the period of the study when compared with control as shown in Fig4.26. The protein level decreased from 37.67 ± 2.10 to 18.75 ± 2.09g/l from day 0 to day 14 and fluctuated for the remaining period of study as indicated by Fig 4.28. However the decrease was significant ($P<0.05$).

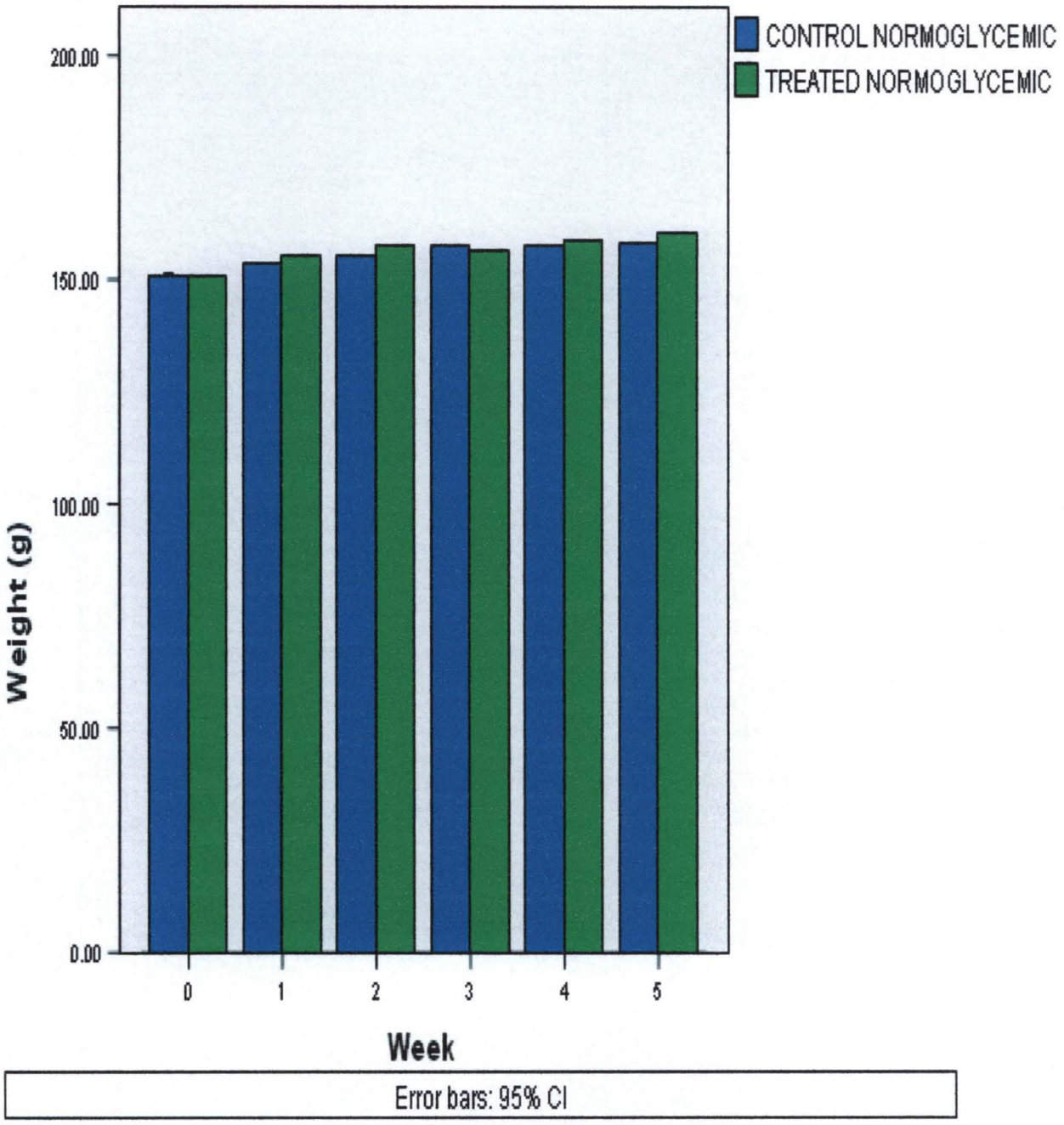


FIG 4.21:Effect of Aqueous extract (500mg/kg bwt) of *M. oleifera* on weight of rats.

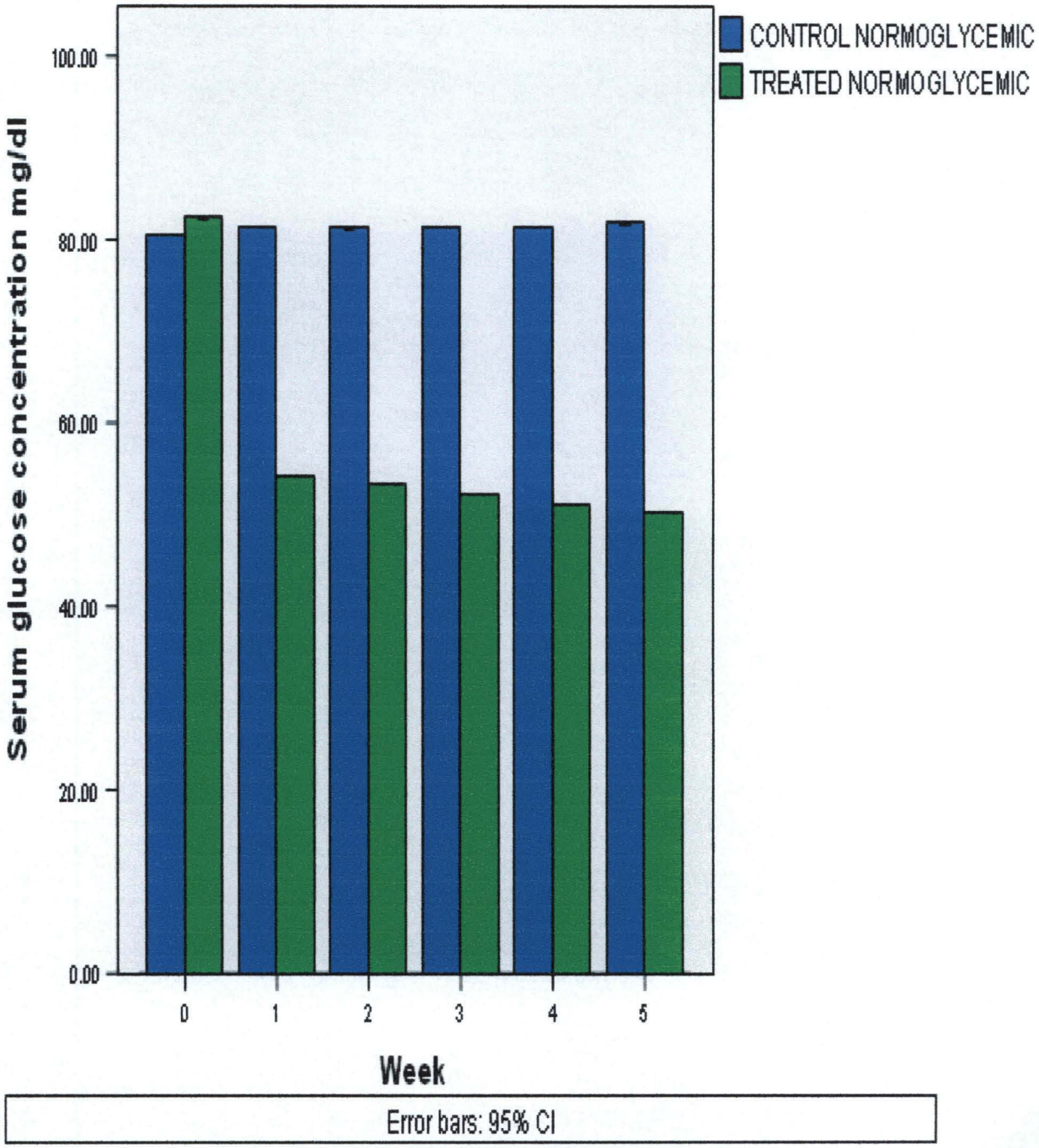


FIG 4.22: Effect of Aqueous extract (500mg/kg bwt) of *Moringa oleifera* on serum glucose concentration in rats.

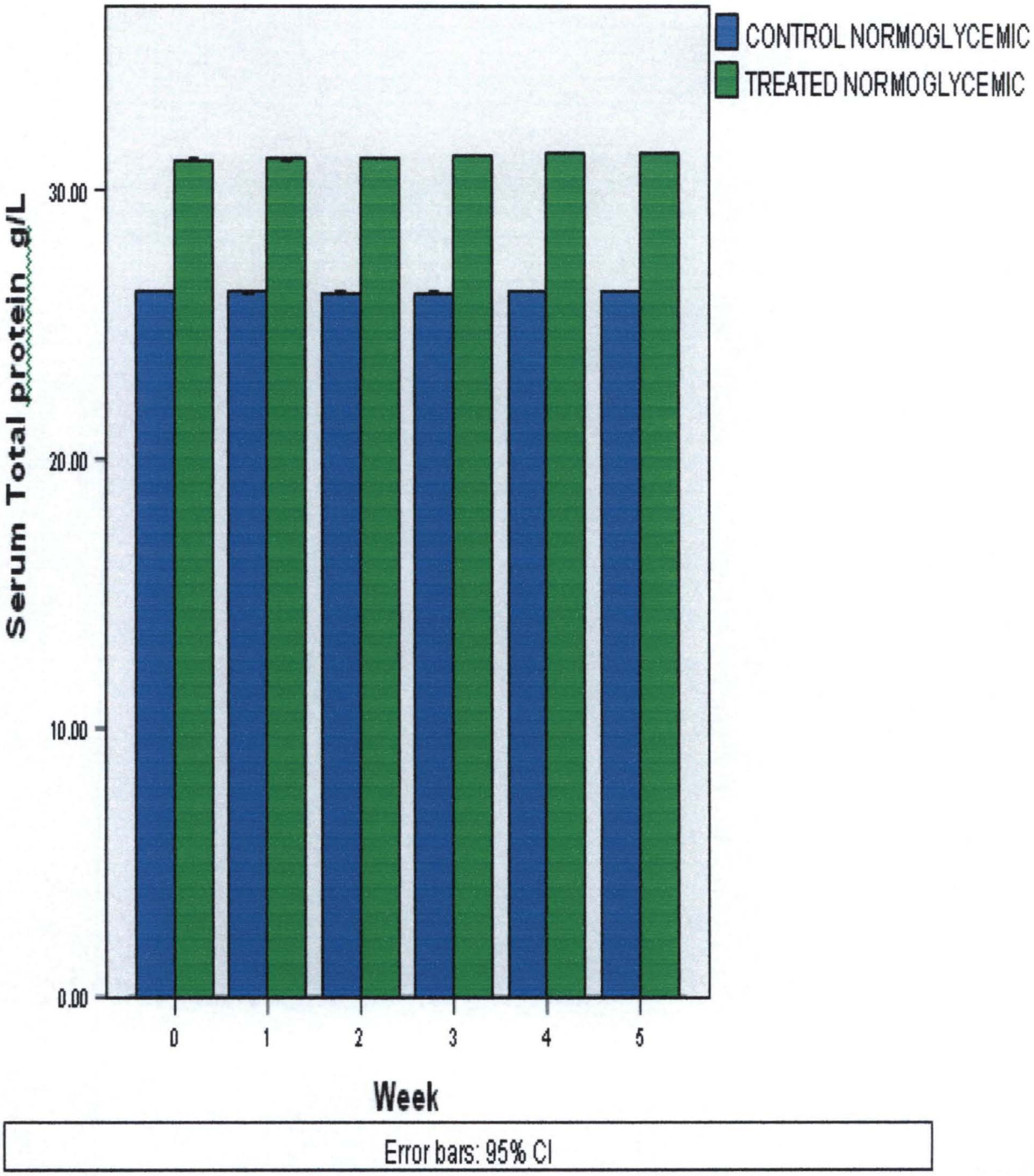


FIG 4.23: Effect of Aqueous extract (500mg/kg bwt) of *M. oleifera* on serum Total protein concentration in rats.

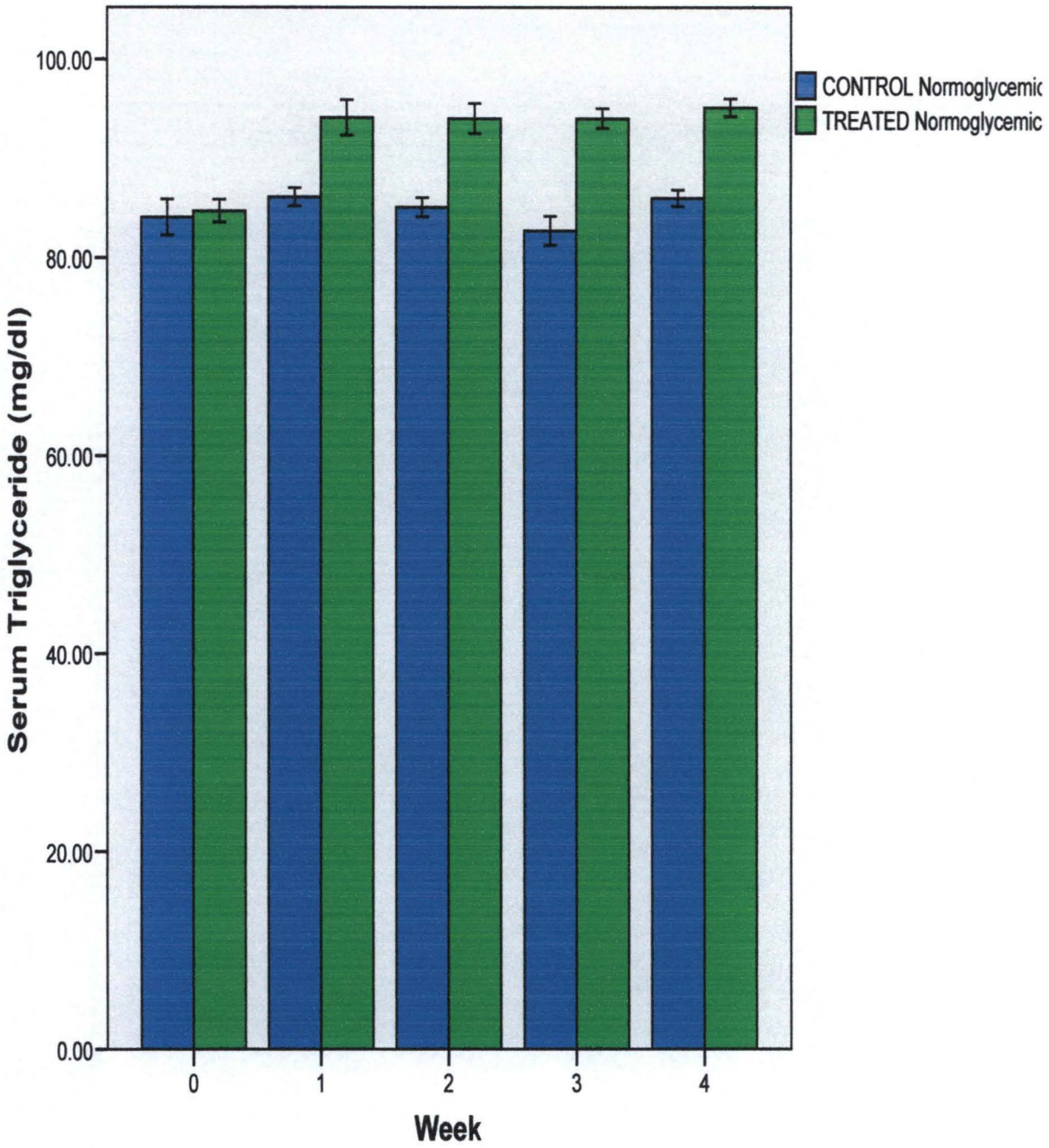


Fig 4.24: Effect of Aqueous extract (500mg/kg bwt) of *Moringa oleifera* on serum triglyceride of rats

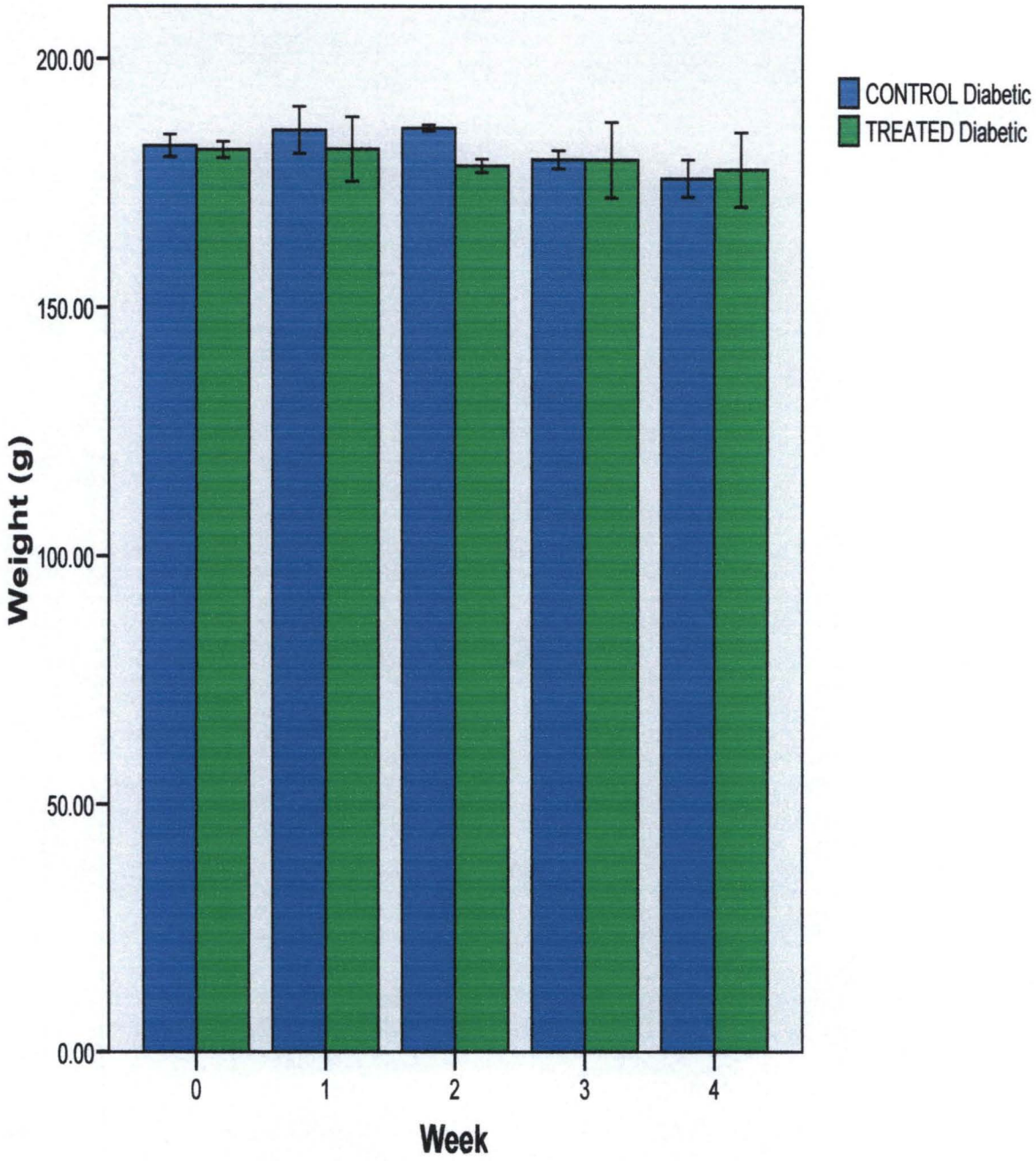


Fig 4.25: Effect of Aqueous extract (500mg/kg bwt) of *Moringa oleifera* on Weight of diabetic rats.

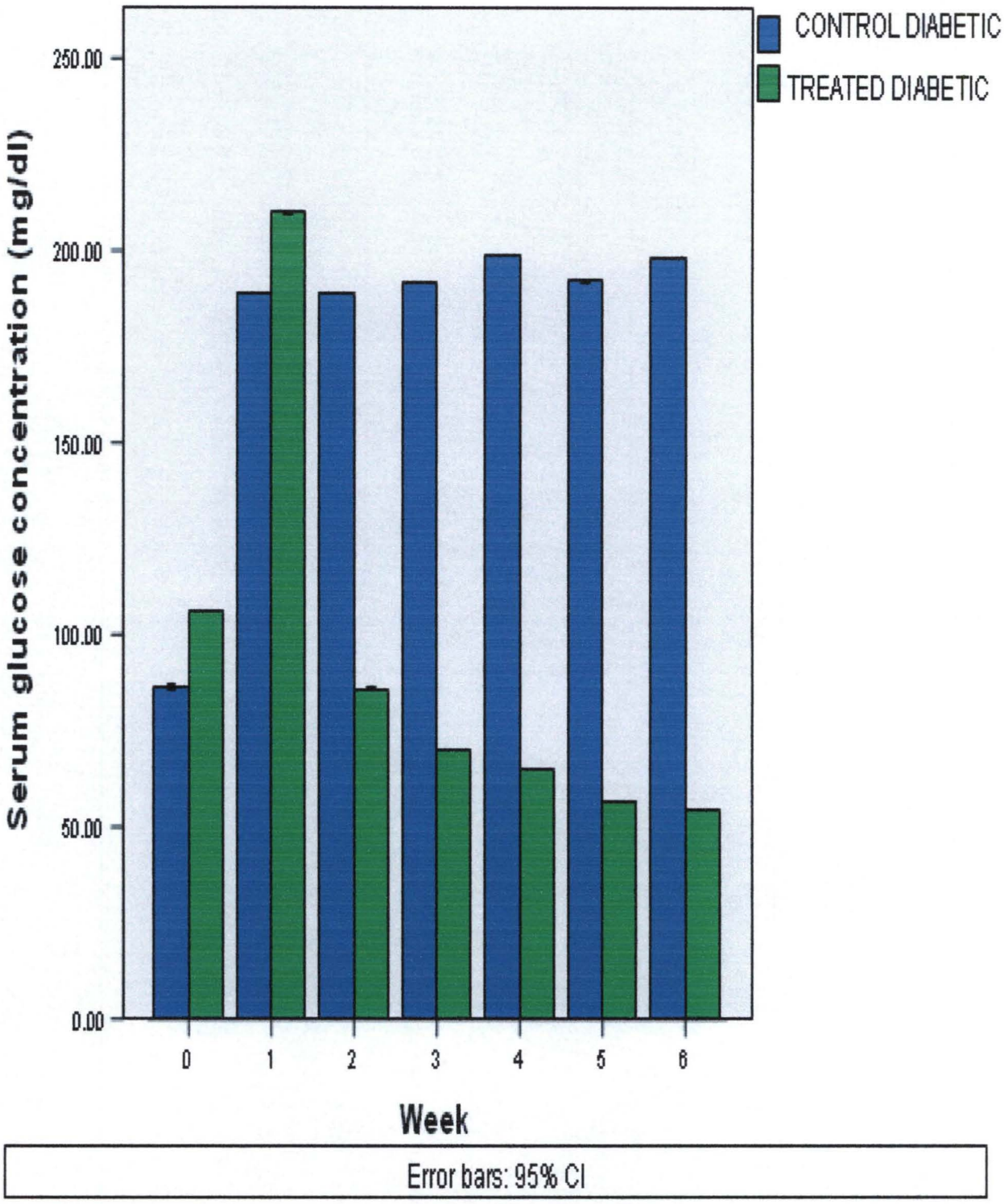


FIG 4.26:Effect of aqueous extract (500mg/Kg b. wt) of *Moringa oleifera* Leaf on serum glucose concentration in dabetic rats

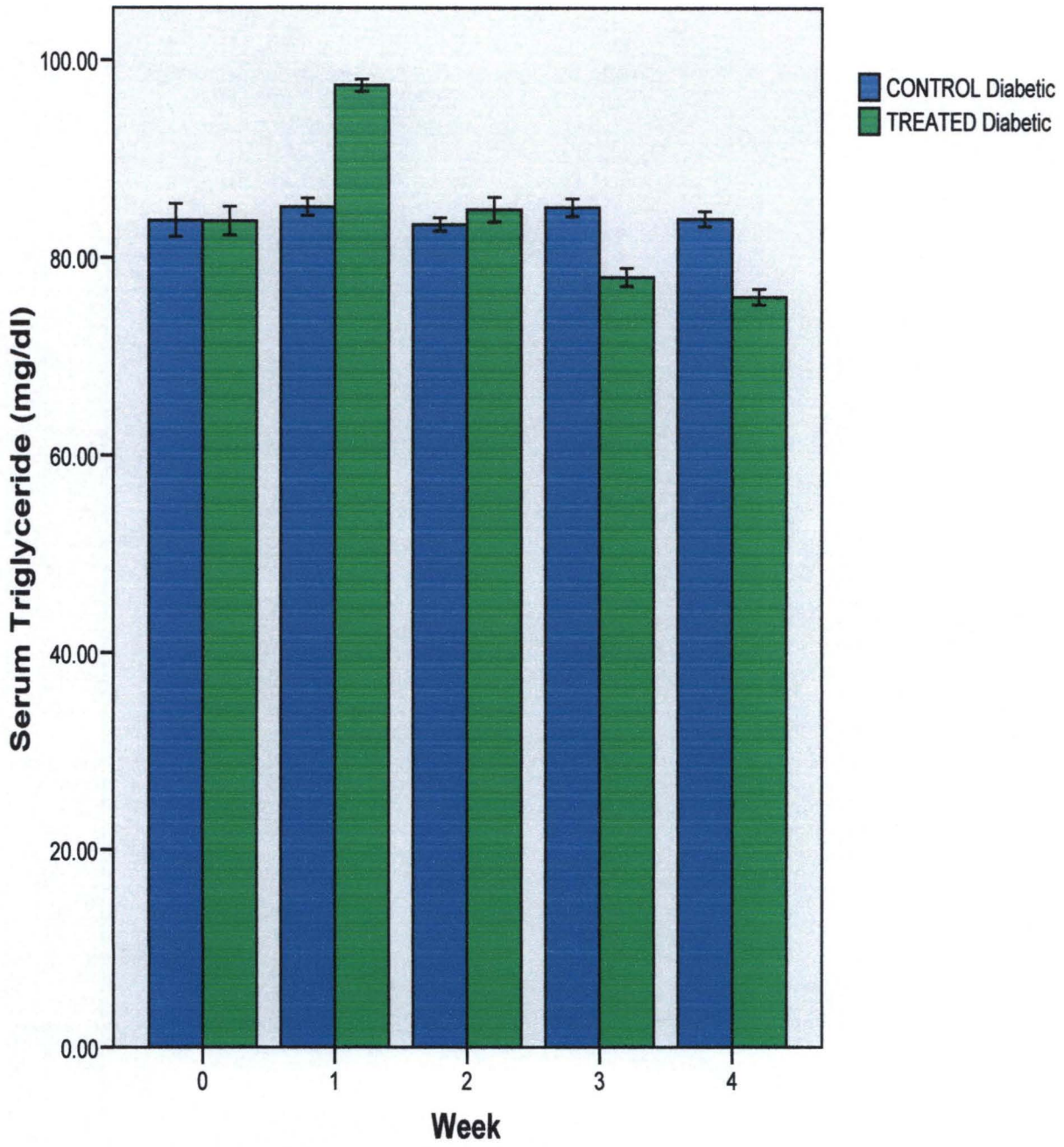


Fig 4.27: Effect of Aqueous extract (500mg/kg bwt) of *Moringa oleifera* on serum triglyceride of diabetic rats.

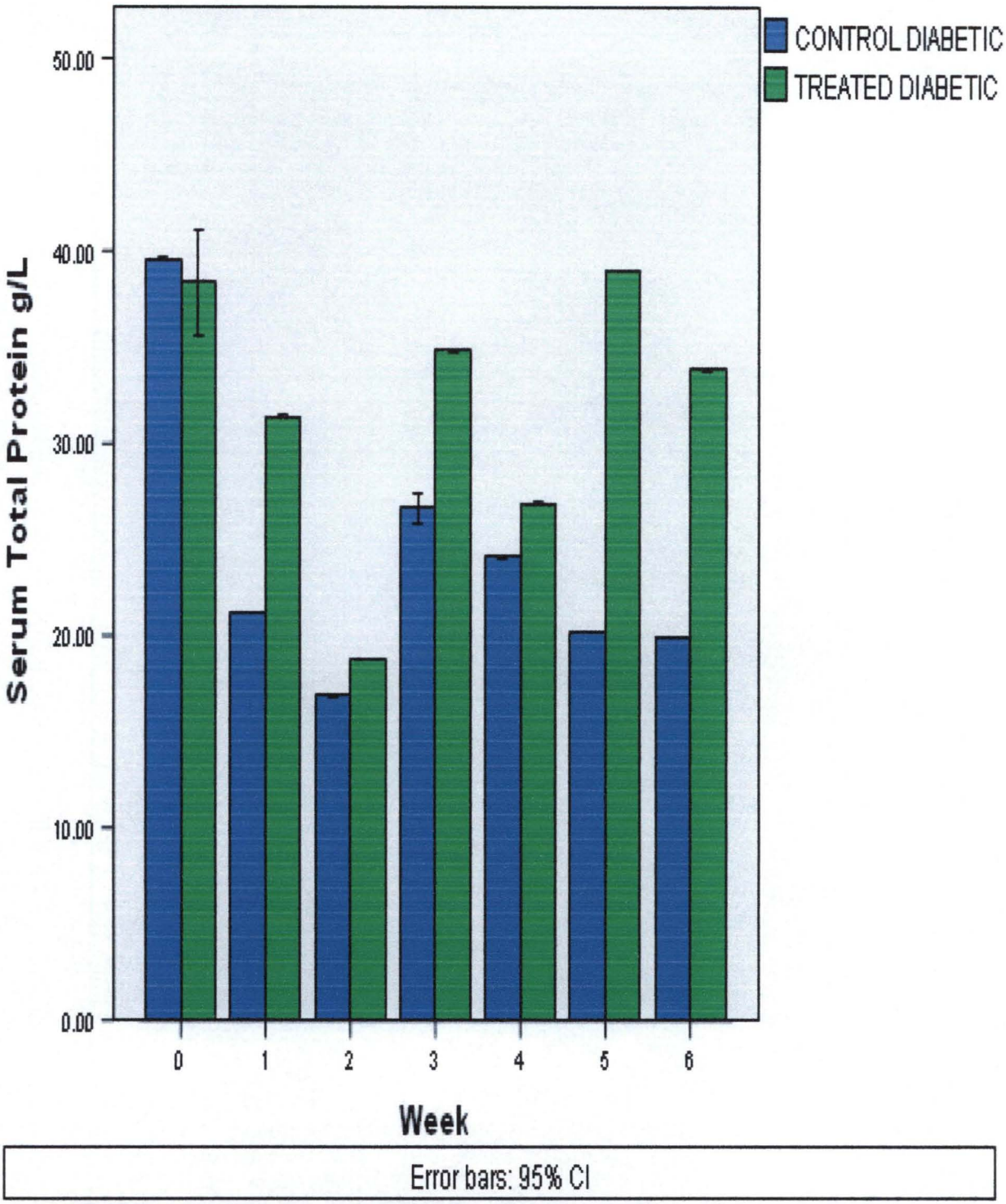


FIG 4.28:Effect of aqueous extract (500mg/kg bwt) of *Moringa oleifera* on serum total protein in diabetic rats.

4.18 Effect of Aqueous Extract of *Zyzzipus spinachristi* Leaf on Serum Glucose Concentration of Diabetic Rats.

The results of the hypoglycemic activities of aqueous extract of *Zyzzipus spinachristis* (900mg/kg bwt) are shown in Fig 4.29. The extract lowered blood glucose (220.10±10.6 to 116.10±3.10mg/dl) from day 0 to day14 and stabilizes for the remaining period of study. However, the decrease was significant ($P<0.05$) when compared with control. There was significant ($P<0.05$) difference between control and diabetic rats.

4.19 Effect of Aqueous Extract of *Artemisia herba-alba* Leaf on Serum Glucose Concentration of Diabetic Rats.

Fig 4.30 showed the hypoglycemic properties of aqueous extract of *Artemisia herba-alba* (525mg/kg bwt). The administration of extract lowered the blood glucose level (240.10 ±13.1 to136.0±12.1mg/dl) from day 0 to day 14 and remains stable for the remaining period of study. The decrease was however significant ($P<0.05$) when compared with control.

4.20 Effect of Aqueous Extract of *Terminalia glaucescens* Leaf on Serum Glucose Concentration of Diabetic Rats.

The results of hypoglycemic properties of aqueous extract of *Terminalia glaucescens* (550mg/kg bwt) are presented in Fig 4.31. The extract lowered the blood glucose levels (230.1± 10.1 to128.10±8.10 mg/dl) from day 0 to day 14 and stabilizes for the remaining period of study. However, the decrease was significant ($P<0.05$) compared with control.

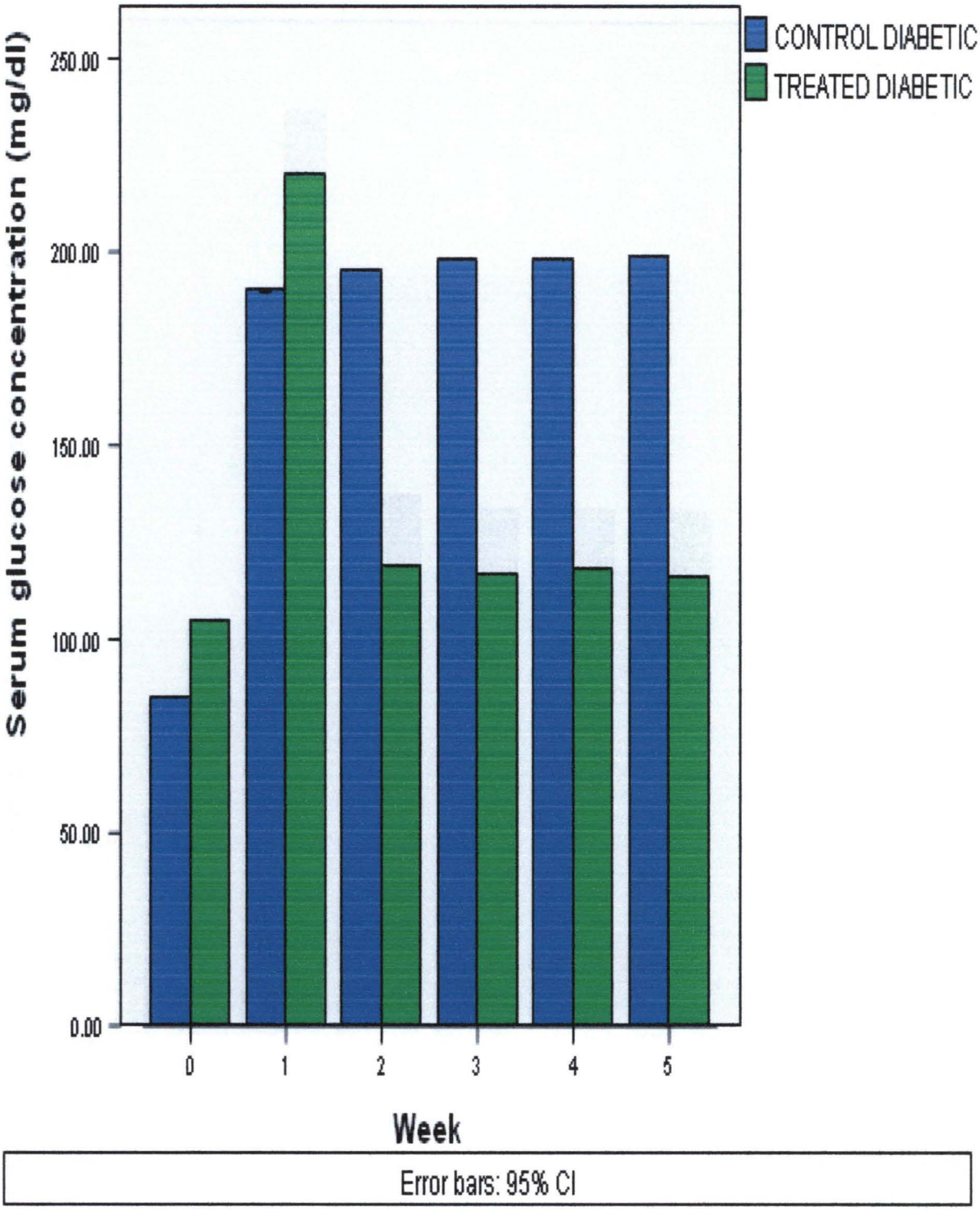


FIG 4.29: Effect of Aqueous extract (900 mg/ Kg .b.wt) of *Zyzzipus spinachristi* on serum glucose concentration in diabetic rats.

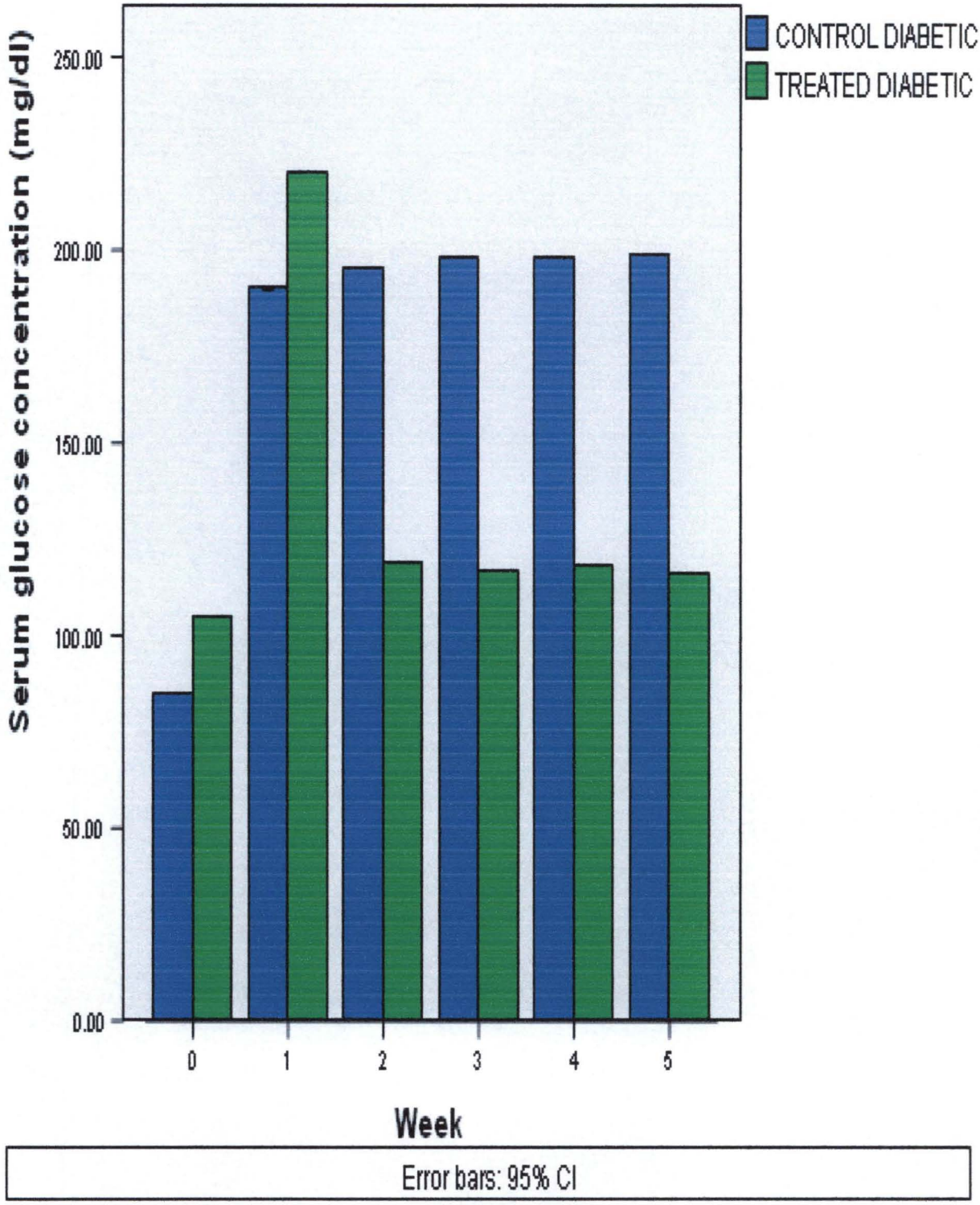


FIG 4.29: Effect of Aqueous extract (900 mg/ Kg .b.wt) of *Zyzzipus spinachristi* on serum glucose concentration in diabetic rats.

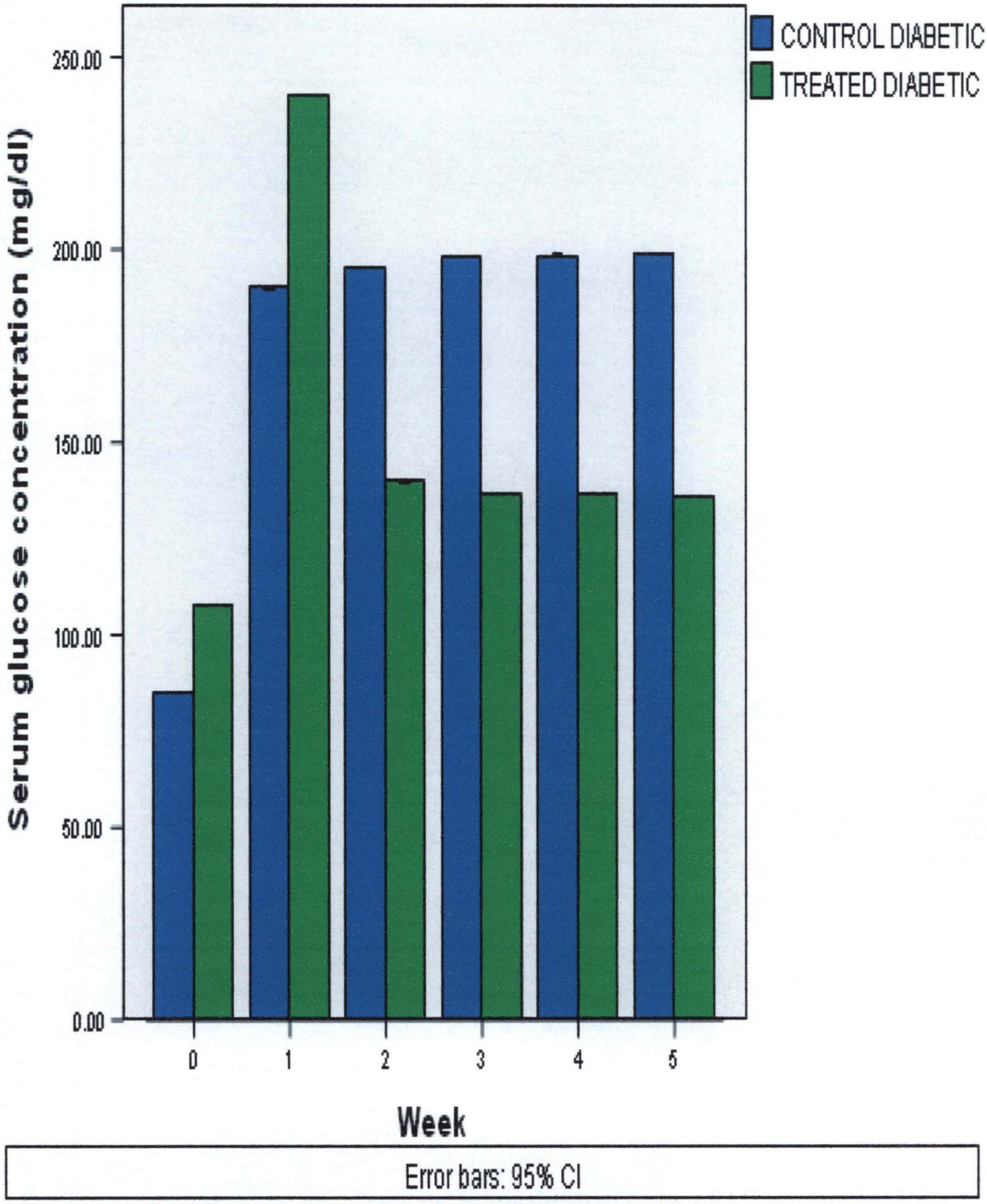


FIG 4.30: Effect of aqueous extract (525mg/ Kg .b.wt) of *Artemisia herba- alba* on serum glucose concentration in diabetic rats.

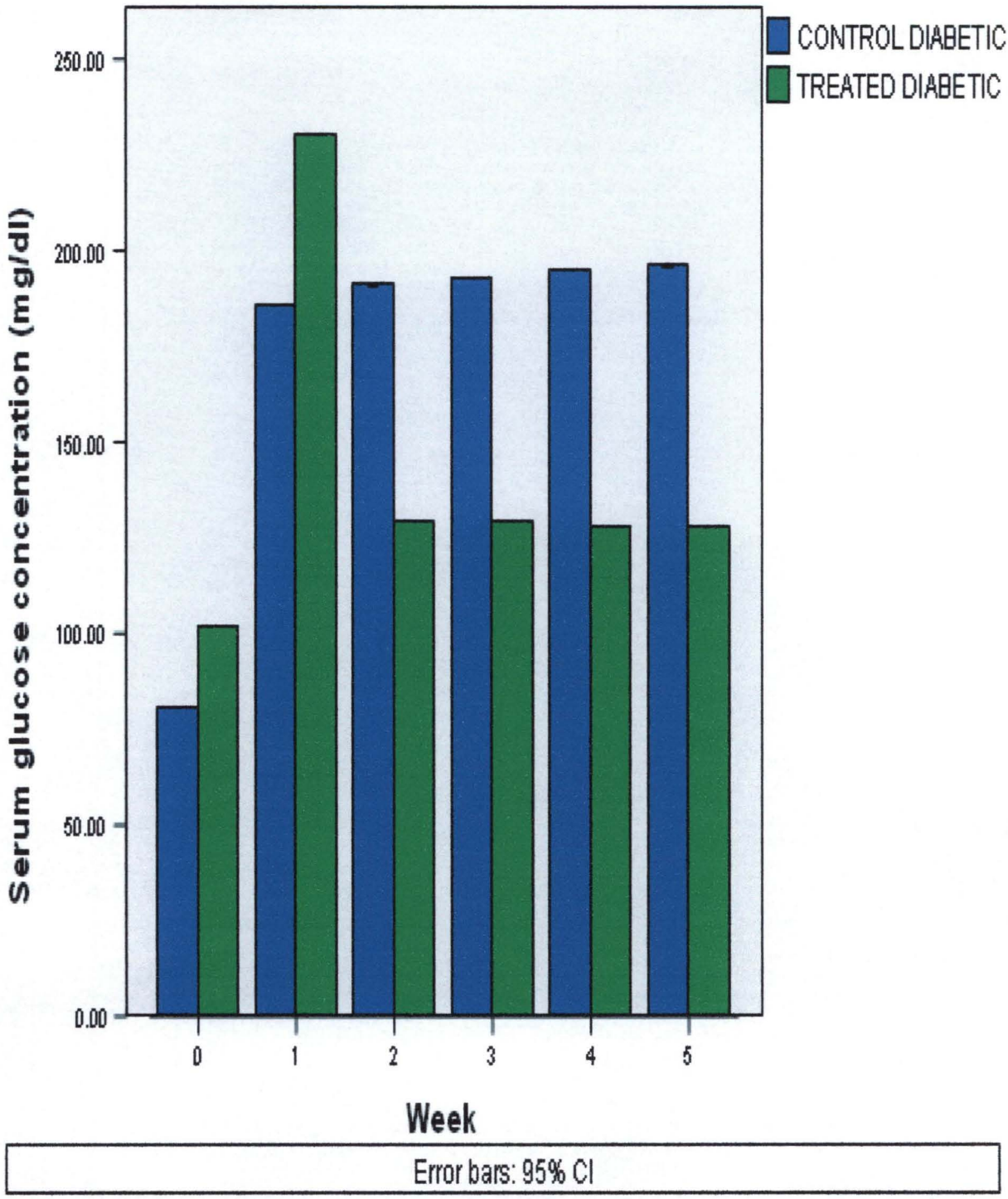


FIG 4.31:Effect of Aqueous extract (550mg/Kg .b. wt) of *Terminalia glaucescens* on serum glucose concentration in diabetic rats

4.21 Effect of Stepwise Fractions of Leaf extract of *Anacardium occidentale* on Serum Glucose Concentration of Diabetic Rats.

The results of the hypoglycemic properties of the stepwise fractions (200mg/kg bwt) of leaf extract of *Anacardium occidentale* are shown in Figs 4.32-4.35. The hexane and ethylacetate/ethanol fractions lowered glucose levels from 183.9±10.2 to 140.1 ±6.10 and from 186.2 ±11.3 to 136.6 ±4.10 mg/dl respectively (Figs 4.32 and 4.34). The ethylacetate and ethanol fractions decreased the glucose levels from 206.1 ±11.1 to 111.6 ±10.1and from 204.8 ±12.2 to 110.1 ±11.6 mg/dl respectively (Figs4.33 and 4.35). All the fractions showed significant ($p<0.05$) decrease compared with control.

4.22 Effect of Stepwise Fractions of stem bark extract of *Anacardium occidentale* on Serum Glucose Concentration of Diabetic Rats.

The results of the hypoglycemic properties of stepwise fractions (200mg/kg bwt) of stem bark extract of *A.occidentale* are shown in Figs 4.36-4.39. The hexane and ethylacetate/ethanol fractions lowered glucose levels from 191.2±7.10 to 188.8 ±6.10mg/dl and from 184.7 ±4.20 to 178.7 ±5.20 mg/dl respectively(Fig4.36-4.38).The ethylacetate and ethanolic fractions decreased glucose levels from 195.8 ±10.9 to 187.5 ±9.10 mg/dl and from 180.6 ± 4.60 to 178.5 ± 5.10 mg/dl respectively (Fig 4.37 and 4.39). However, the decrease was non-significant ($P>0.05$) compared with control.

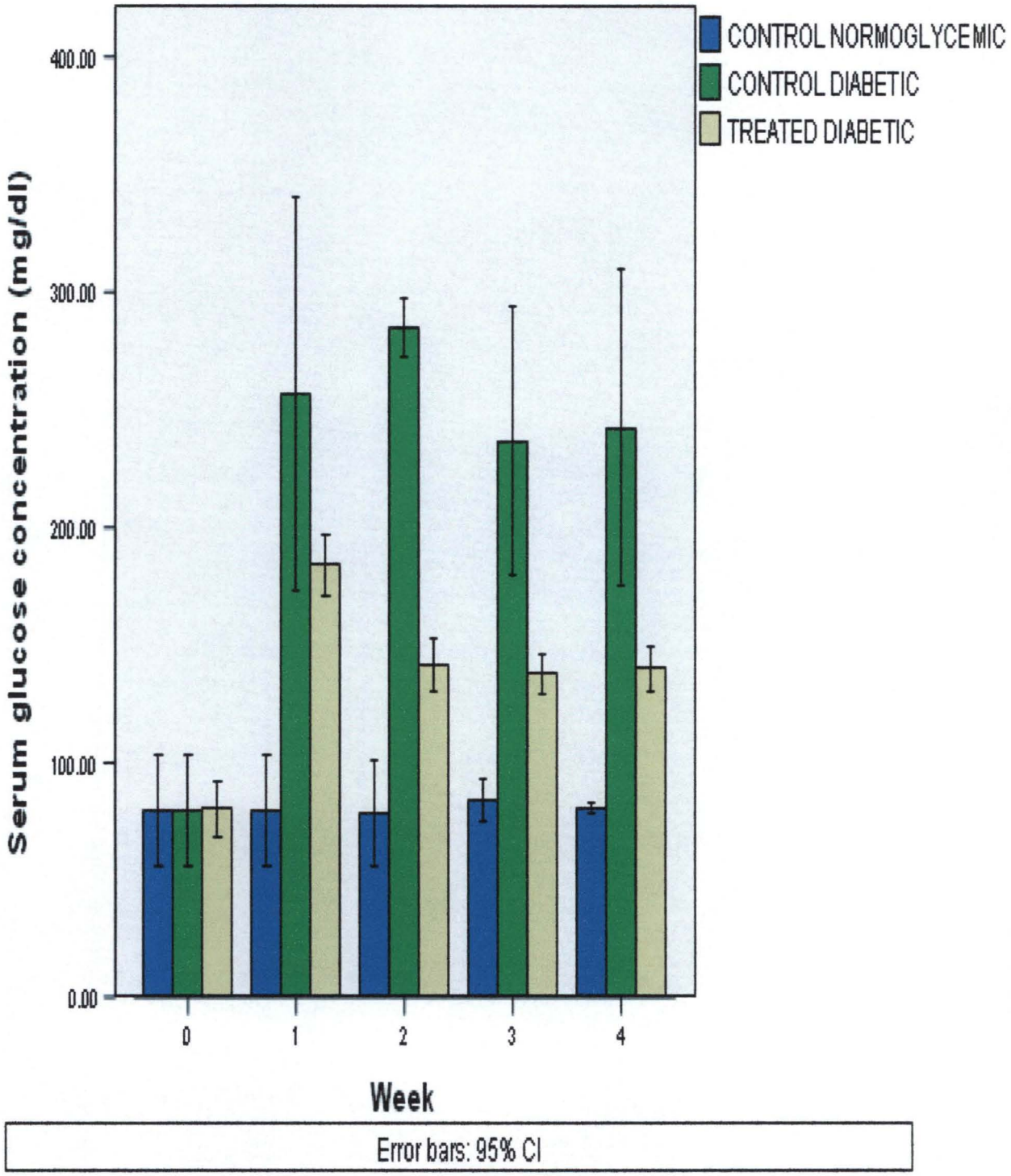


FIG 4.32:Effect of Hexane fraction(200mg / Kg . b. wt) of leaf extract of *A. occidentale* on serum glucose concentration in diabetic rats.

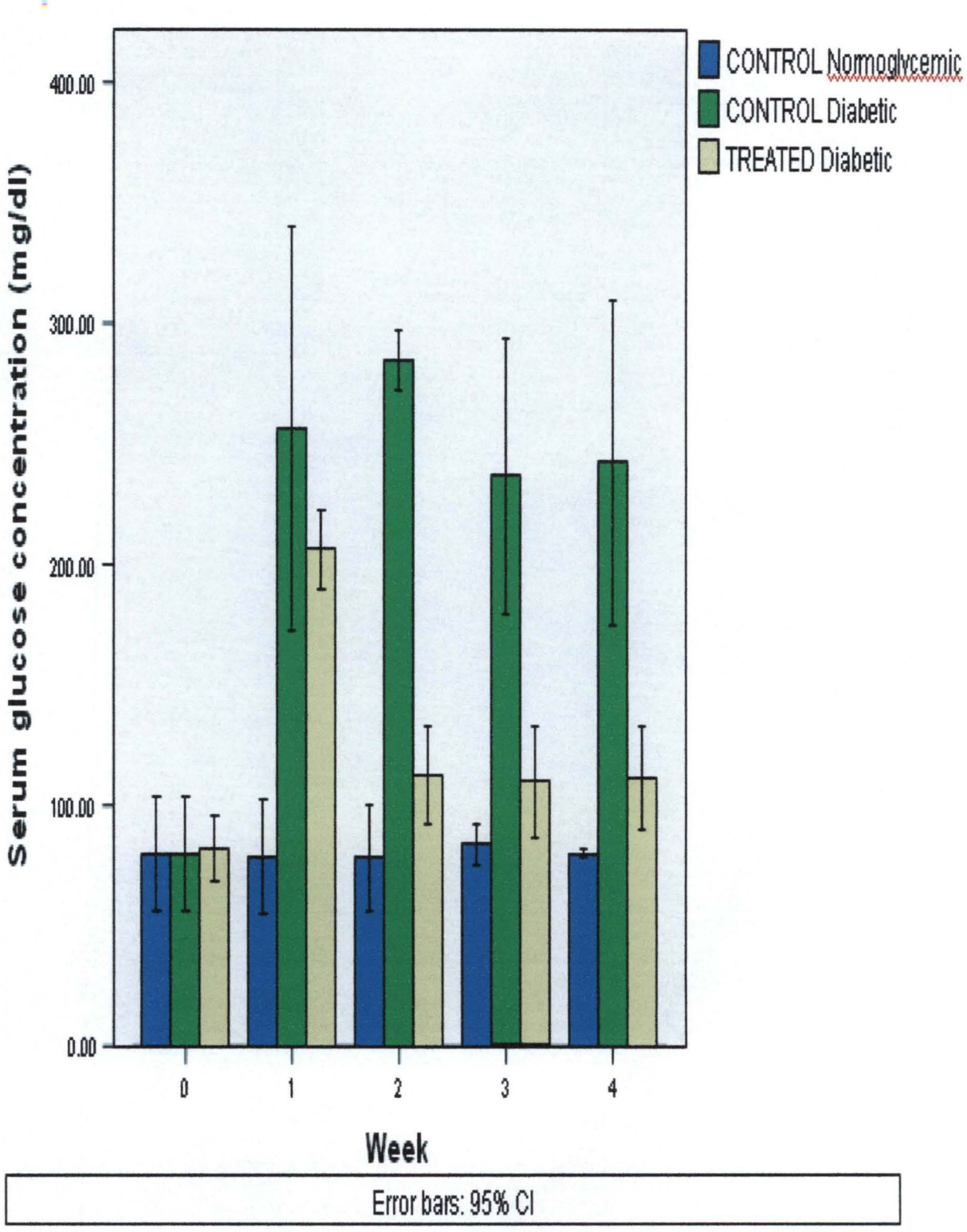


FIG 4.33:Effect of Ethylacetate fraction (200mg/kg bwt) of leaf extract of *A occidentale* on serum glucose concentration in diabetic rats.

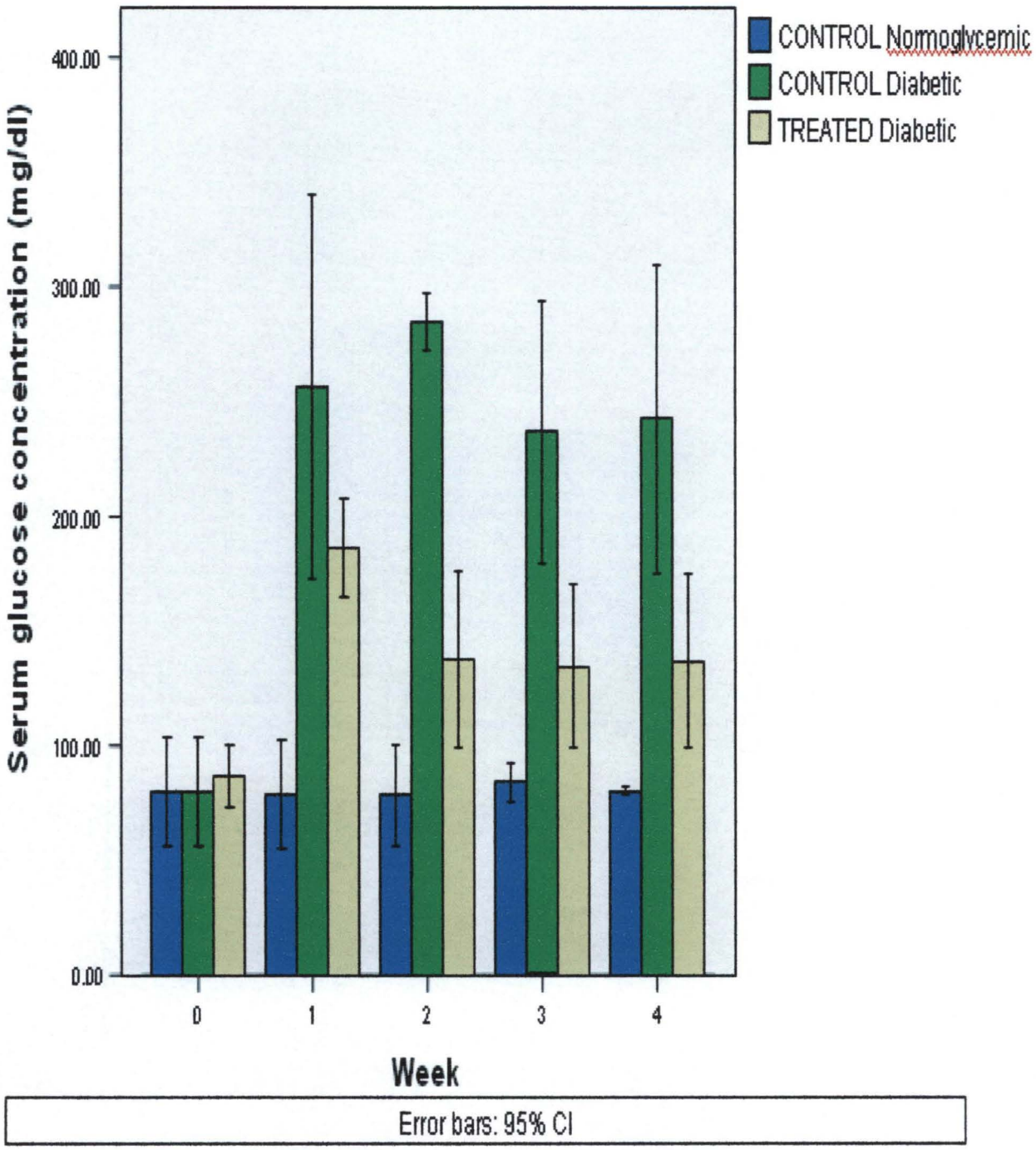


FIG 4.34: Effect of Ethylacetate/Ethanol fraction [200mg/Kg.b. wt] of leaf extract of *A. occidentale* on serum glucose concentration in diabetic rats.

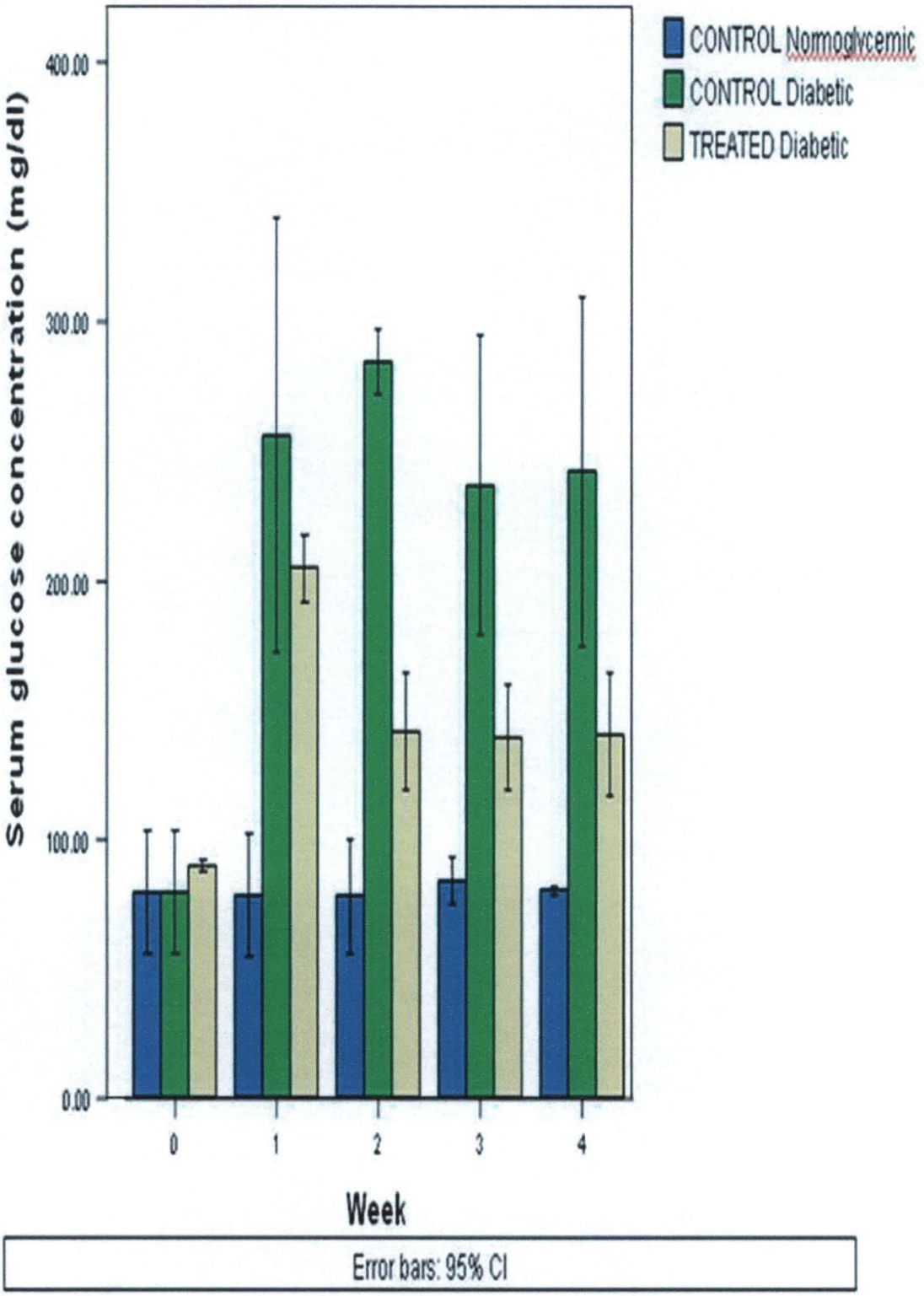


FIG 4.35: Effect of Ethanol fraction (200mg/kg.b.wt) of leaf extract of *A. occidentale* on serum glucose concentration in diabetic rats.

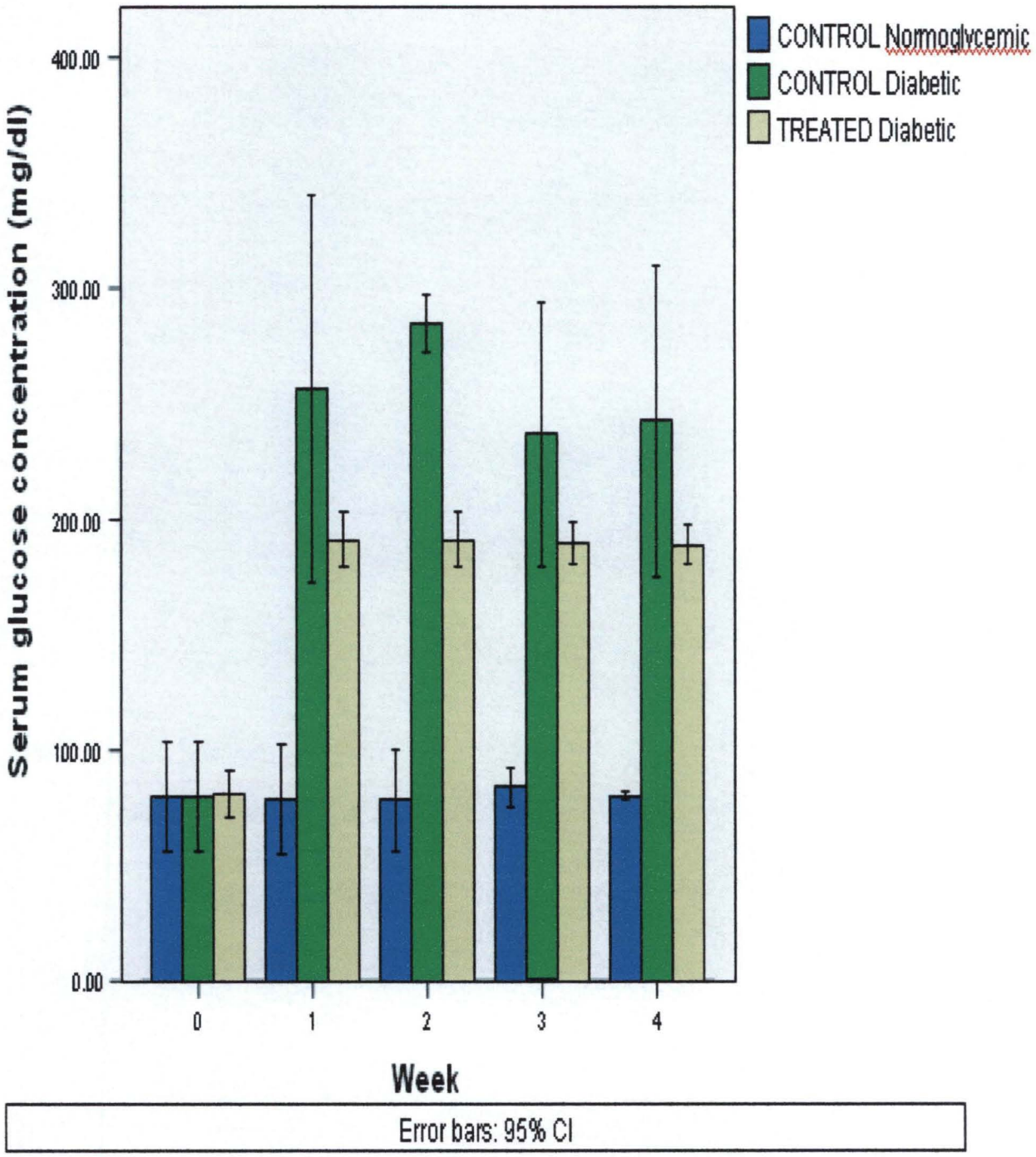


FIG 4.36: Effect of Hexane fraction(200mg/Kg. b.wt) of stem bark extract of *A. occidentale* on serum glucose concentration in diabetic rats

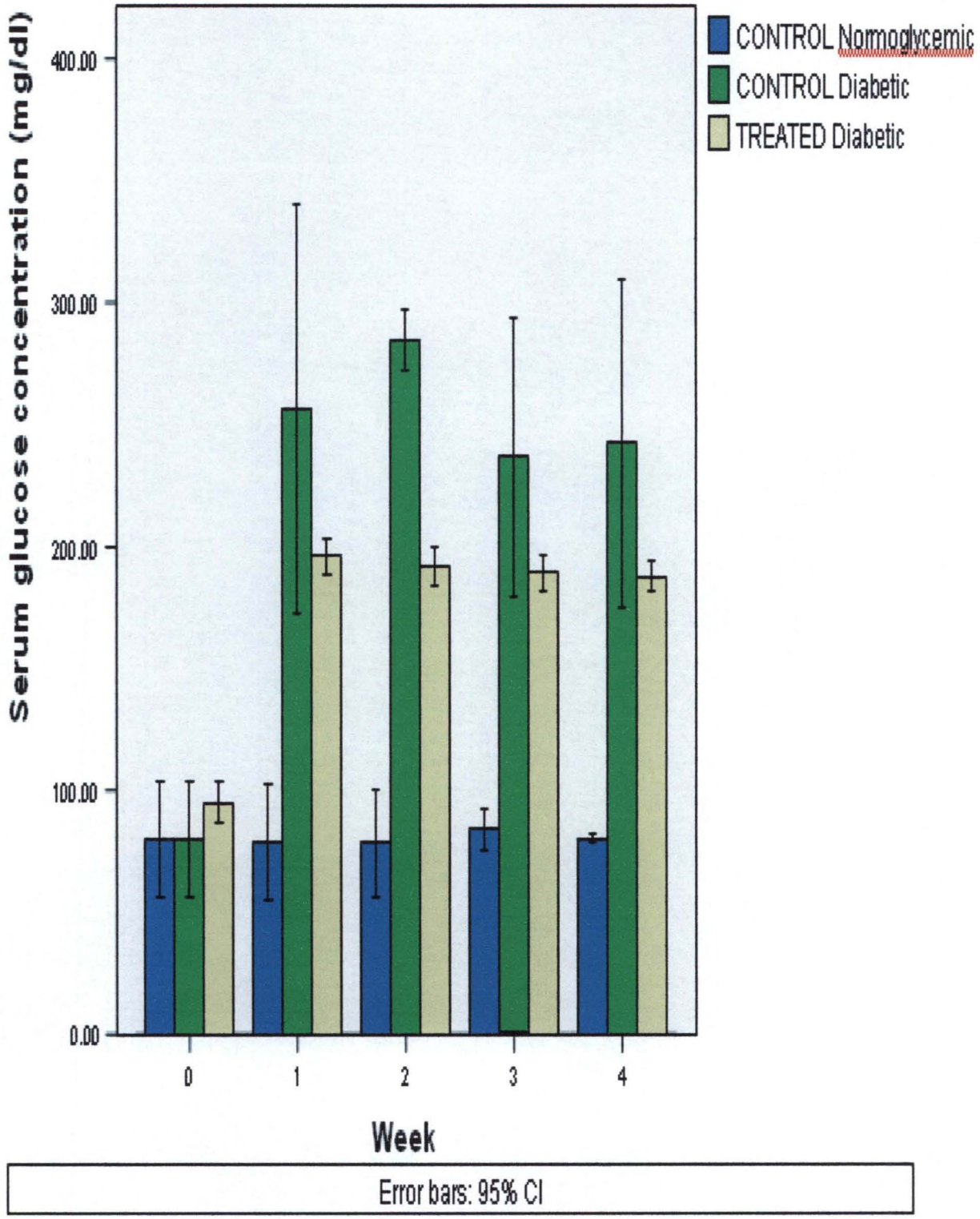


FIG 4.37: Effect of Ethylacetate fraction(200mg/kg .b.wt) of stem bark extract of *A. occidentale* extract on serum glucose concentration in diabetic rats.

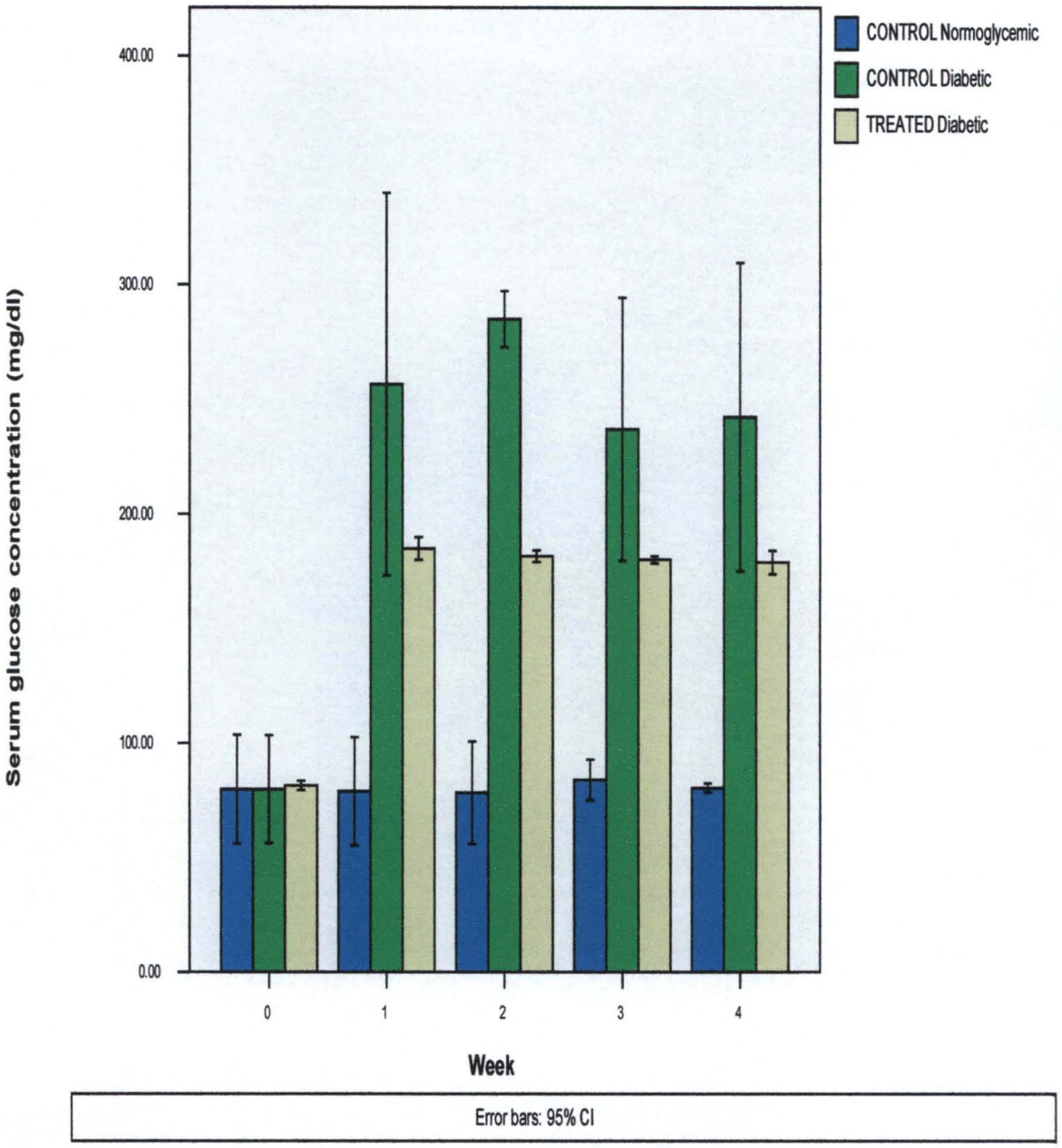


FIG 4.38:Effect of Ethylacetate/Ethanol fraction[200mg/kg bwt] of stem bark extract of *A .occidentale* on serum glucose concentration in diabetic rats.

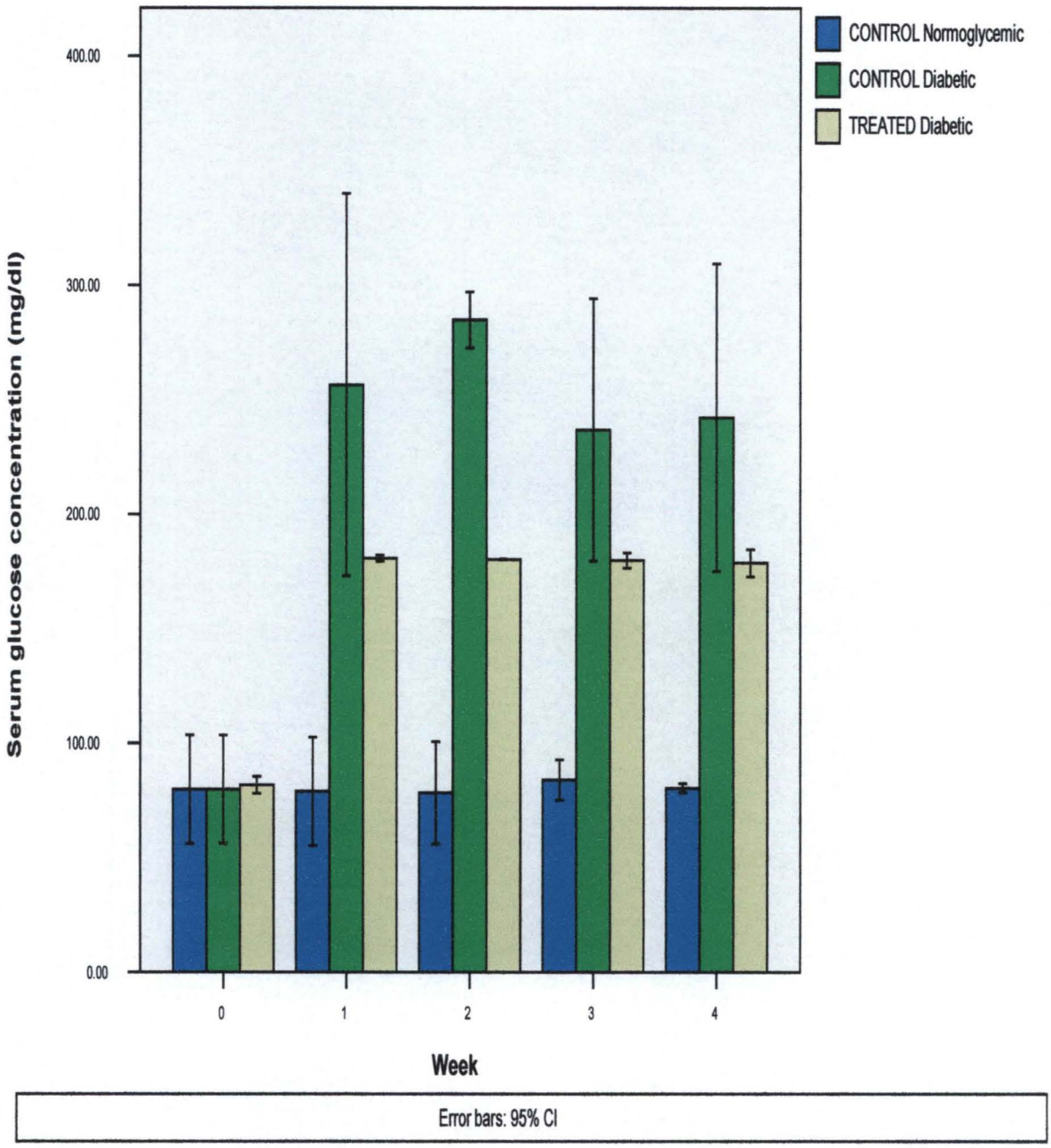


FIG 4.39:Effect of ethanol fracion (200mg/Kg .b.wt) of stem bark extract of *A. occidentale* on serum glucose concentration in diabetic rats.

4.23 Effect of Stepwise Fractions of leaf extract of *Anacardium occidentale* on Serum Glucose Concentration of Diabetic Rats.

The results of the hypoglycemic activities of stepwise fractions (300mg/kg bwt) of leaf extract of *A. occidentale* are shown in Figs 4.40-4.43. The hexane and ethylacetate/ethanol fractions decreased glucose levels from 193.8±12.1 to192.5±11.9 mg/dl and from 190.5±12.6 to 186.6±11.2mg/dl respectively(Figs 4.40 and 4.42). The decrease was however not significant (P>0.05) compared with control.The ethylacetate and ethanol fractions lowered glucose levels from 179.2 ±11.2 to 143.2±9.10mg/dl and from 185.2±6.20 to147.7 ±5.20 mg/dl respectively (Figs 4.41 and 4.43). However, the decrease was significant (P<0.05) compared with control.

4.24 Effect of Stepwise Fractions of stem bark extract of *Anacardium occidentale* on Serum Glucose Concentration of Diabetic Rats.

The results of the hypoglycemic properties of stepwise fractions (300mg/kg bwt) of stem bark extract of *A.occidentale* are shown in Figs 4.44-4.47. The hexane fraction decreased glucose levels from 193.1±6.21 to 190.5±10.1mg/dl as shown in Fig 4.44. However, the decrease was not significant (P>0.05) compared with control. The ethylacetate, ethylacetate/ethanol and ethanol fractions lowered glucose levels from 195.3±10.2 to 186.7±7.10mg/dl, 190.6±10.1 to 182.8±9.21mg/dl and from 194.1±11.2 to 185.6±8.11 mg/dl respectively (Fig 4.45-4.47). The decrease was however significant (P<0.05) compared with control,

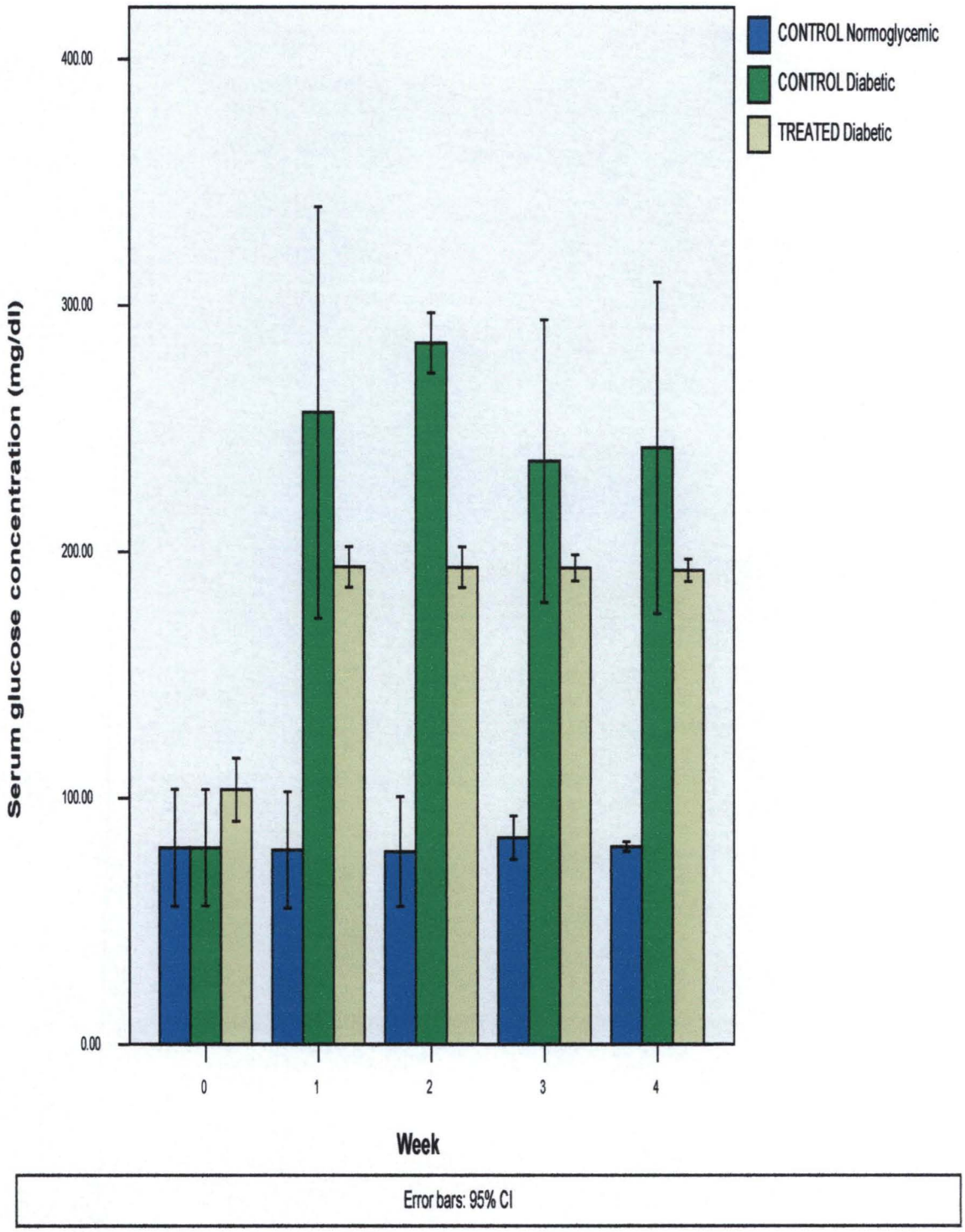


FIG 4.40:Effect of Hexane fraction(300mg/Kg b.wt) leaf extract of *A . occidentale* on serum glucose concentration in diabetic rats.

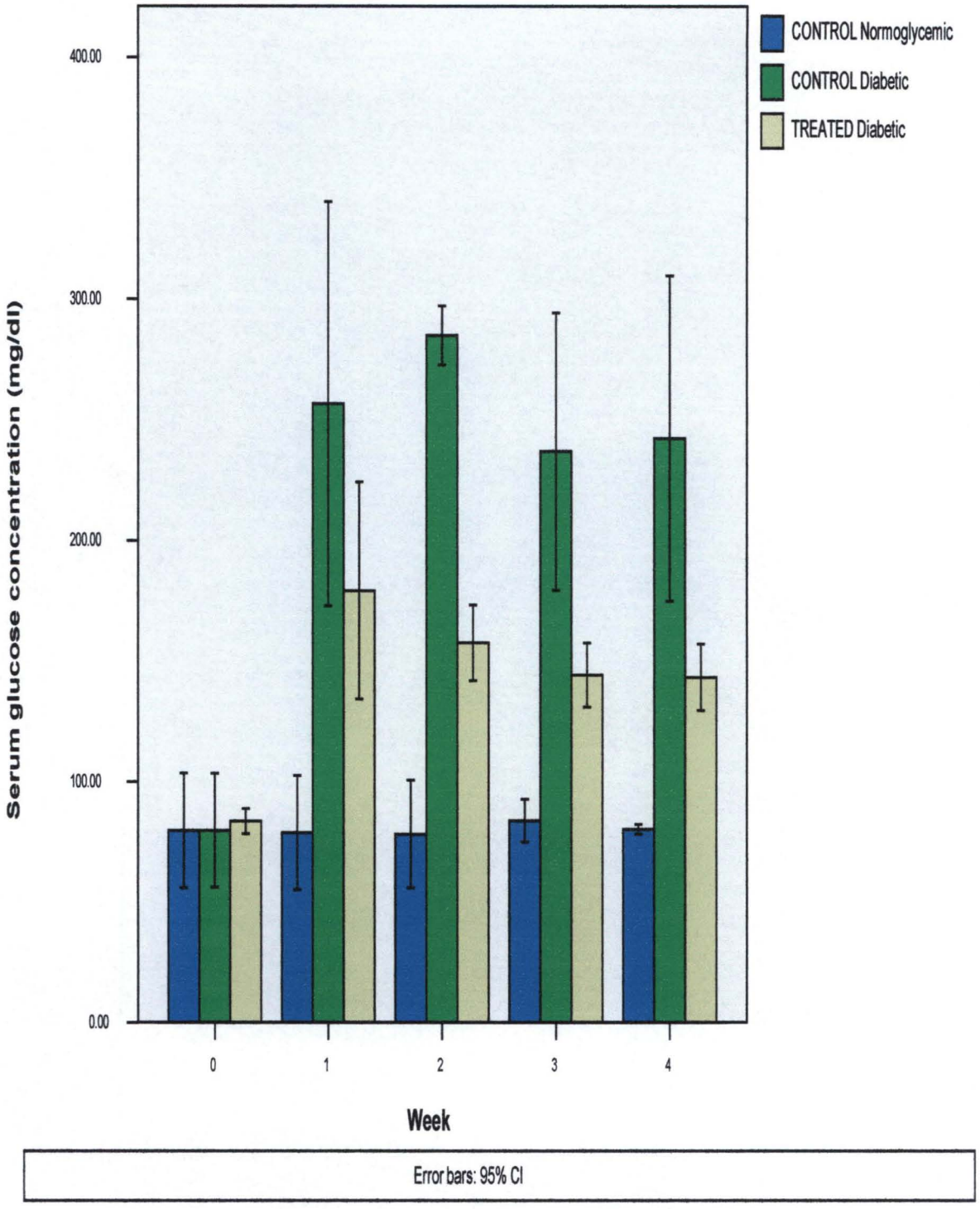


FIG4. 41: Effect of ethylacetate fraction(300mg/kg. b.wt) of leaf extract of *A. occidentale* on serum glucose concentration in diabetic rats.

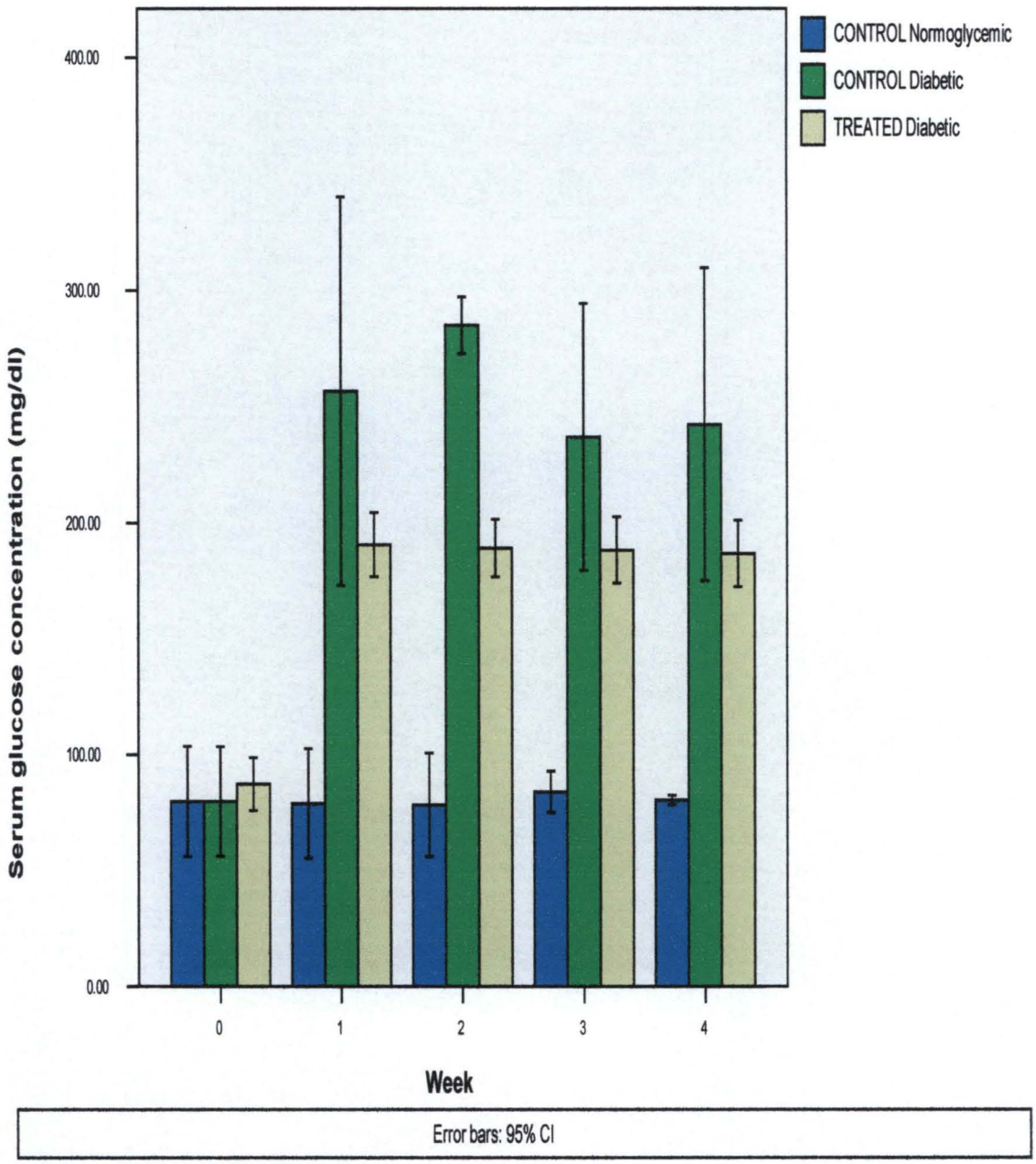


FIG 4.42: Effect of ethylacetate/ ethanol fraction [300mg/kg .b.wt] of leaf extract of *A. occidentale* on serum glucose concentration in diabetic rats.

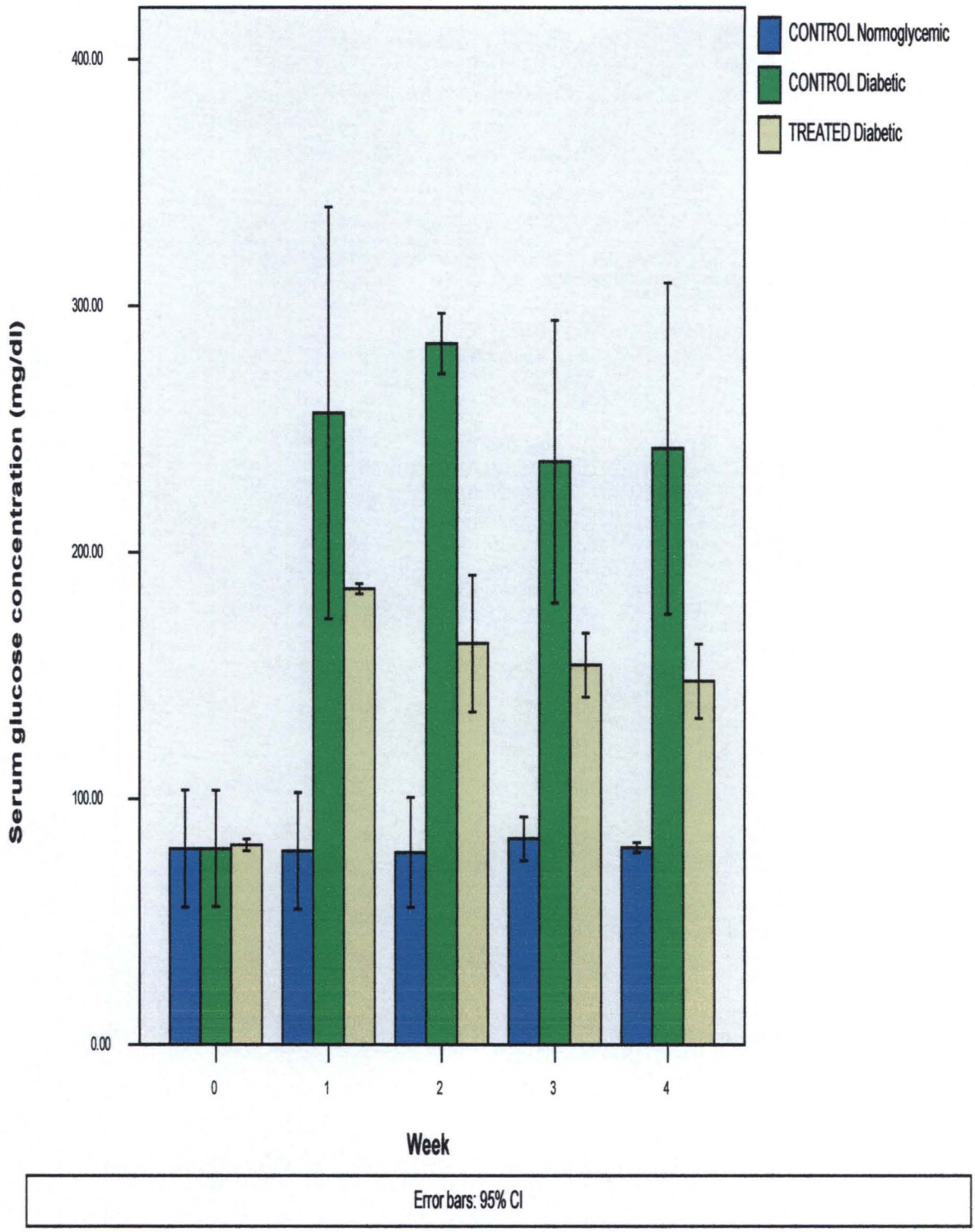


FIG4.43:Effect of ethanol fraction (300mg/kg .b.wt) of leaf extract of *A. occidentale* on serum glucose concentration in diabetic rats.

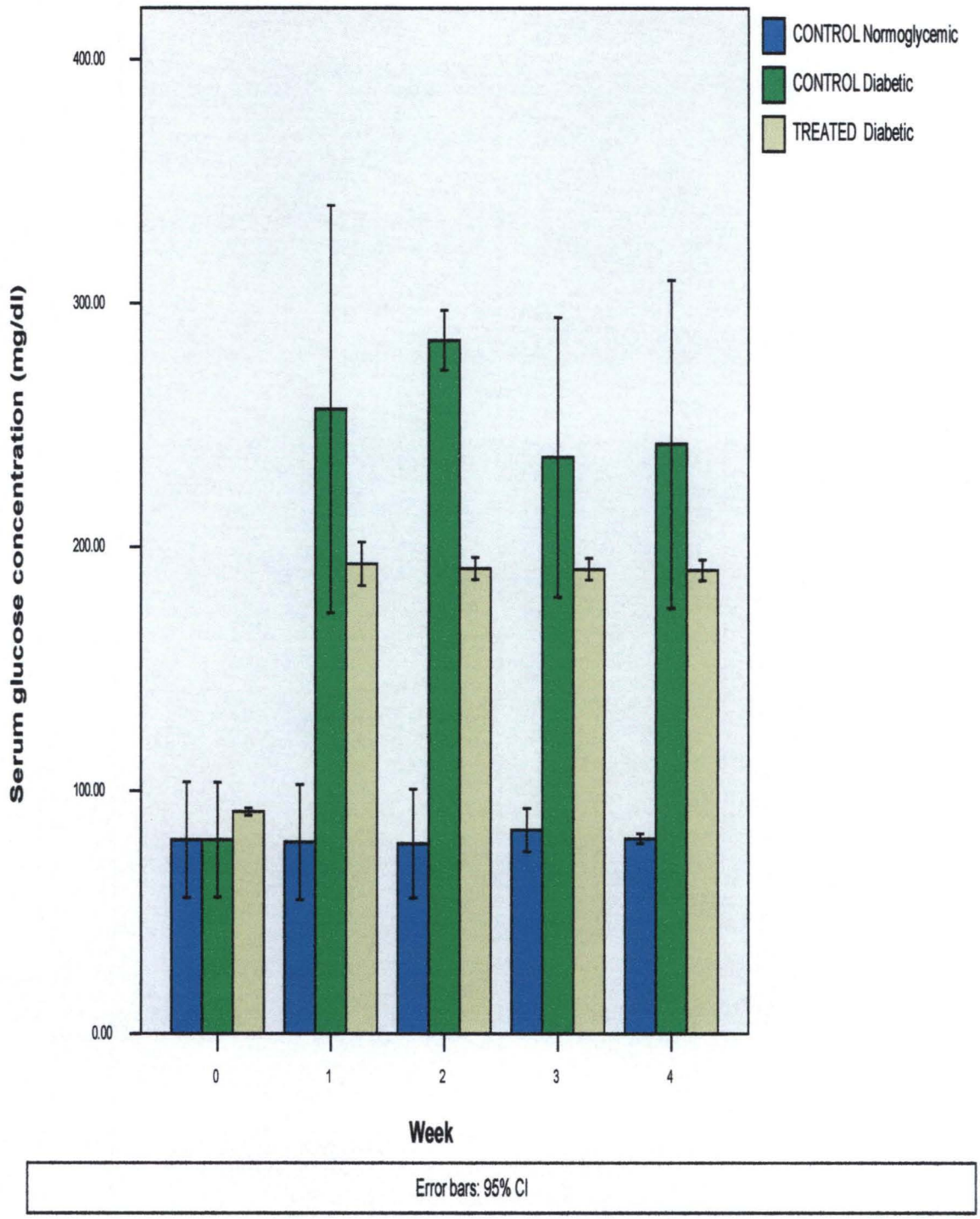


FIG 4.44:Effect of Hexane fraction (300mg/kg bwt) stem bark extract of *A occidentale* on serum glucose concentration in diabetic rats.

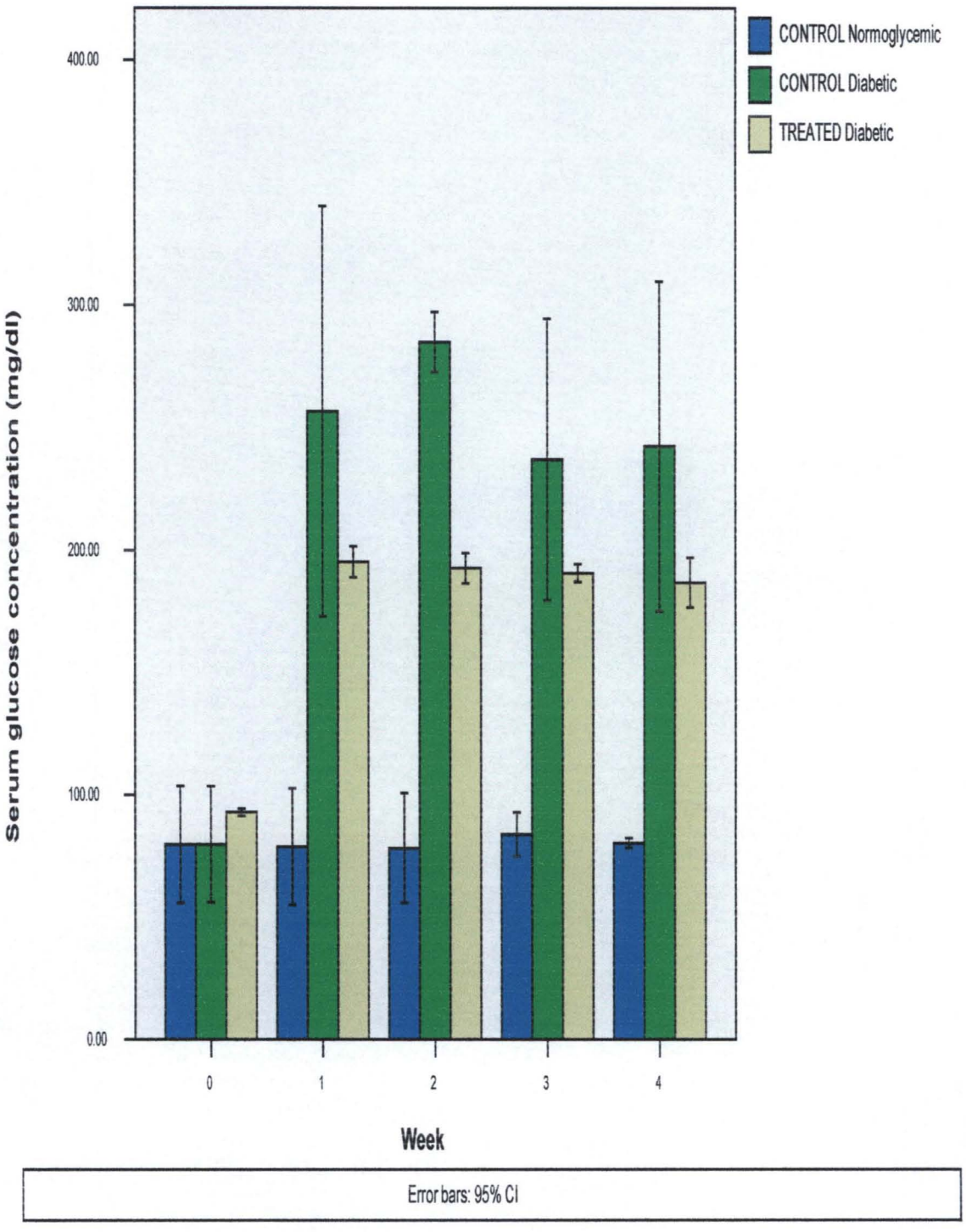


FIG 4.45: Effect of ethylacetate fraction(300mg/kgbwt) of stem bark extract of *A. occidentale* on serum glucose concentration in diabetic rats.

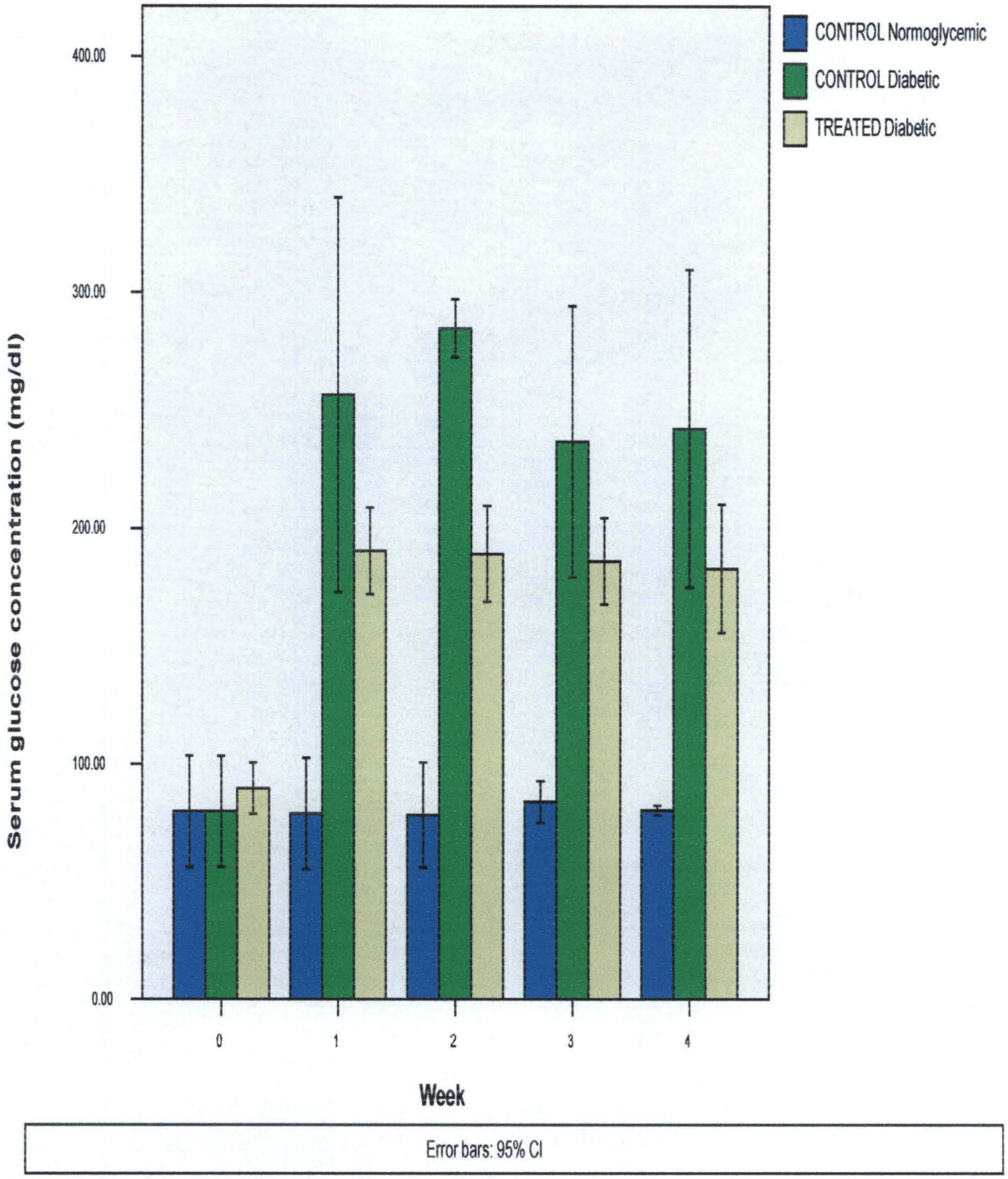


FIG 4.46; Effect of ethylacetate/ethanol fraction [300mg/kg.b.wt] of stem bark extract of *A. occidentale* on serum glucose concentration in diabetic rats.

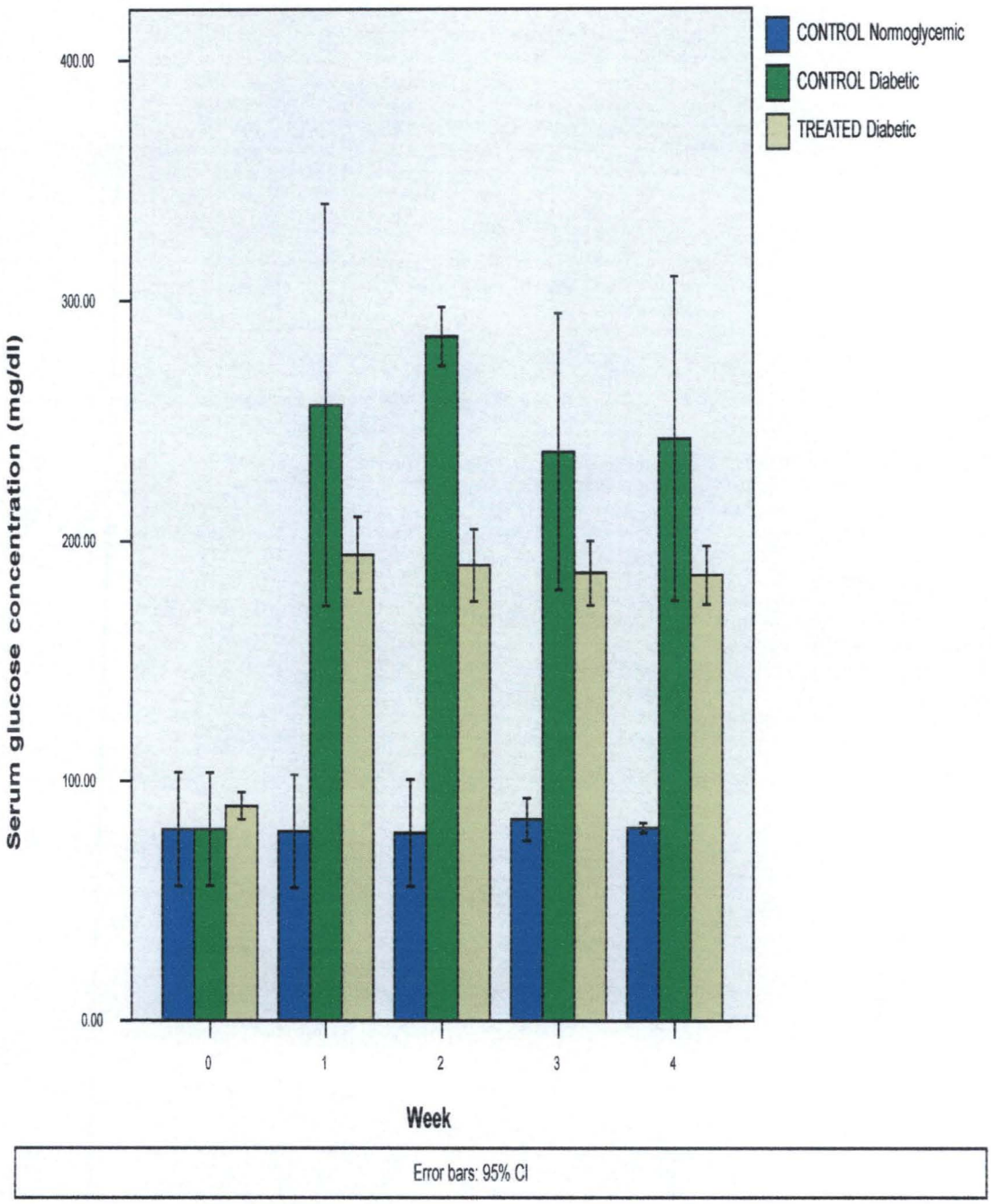


FIG 4.47: Effect of ethanol fraction(300mg/kg .b.wt) of stem bark extract of *A. occidentale* on serum glucose concentration in diabetic rats.

4.25 Effect of Stepwise Fractions of leaf extract of *Anacardium occidentale* on weight of Diabetic Rats.

300mg/kg bwt

The results of the hypoglycemic activities of stepwise fractions (300mg/kg bwt) of leaf extract of *A. occidentale* are depicted in Figs 4.48-4.51. All the fractions non significantly (P>0.05) decreased the weight of rats compared to control as shown in Figs 4.48- 4.51 respectively.

200mg/kg bwt

The results of the hypoglycemic activities of stepwise fractions (200mg/kg bwt) of leaf extract of *A. occidentale* are shown in Figs 4.52-4.55. All the fractions non significantly (P>0.05) decreased the weight of rats compared with control as depicted in Figs 4.52- 4.55 respectively.

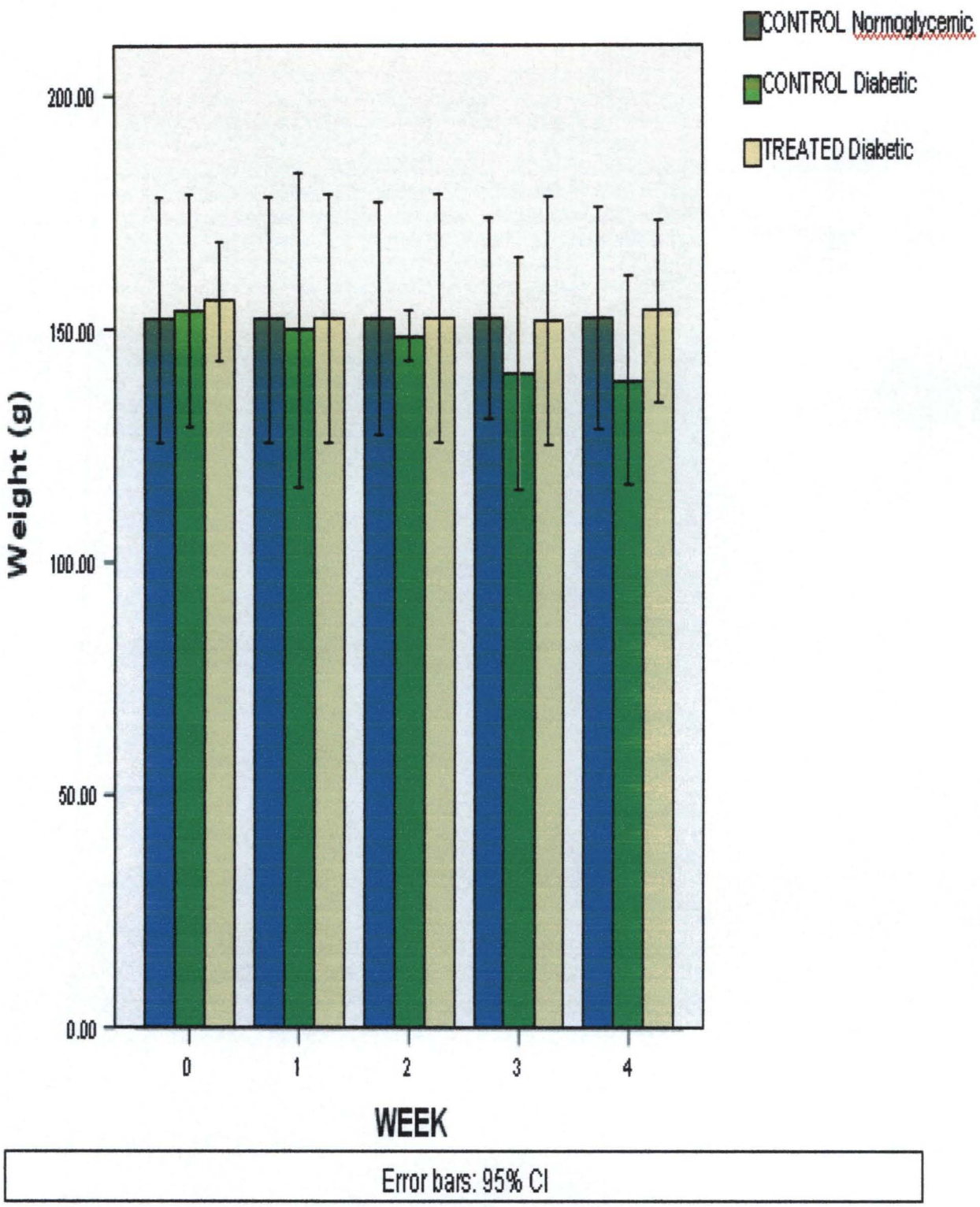


FIG 4.48: Effect of Hexane fraction(300mg/kg b. wt) of leaf extract of *A. occidentale* on weight of diabetic rats.

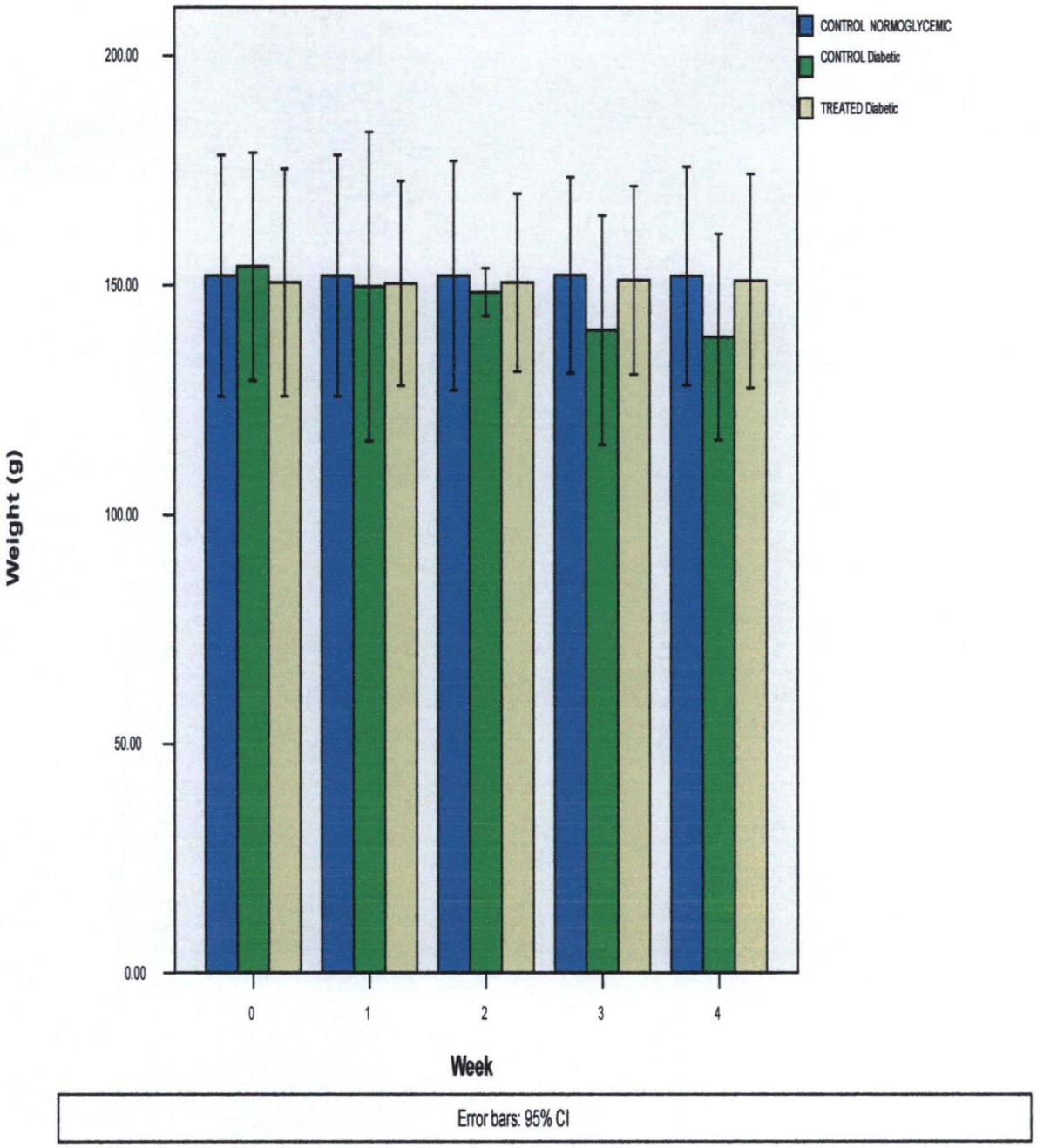


FIG 4.49: Effect of ethylacetate fraction(300mg/kg .b.wt) of leaf extract of *A. occidentale* on weight of diabetic rats.

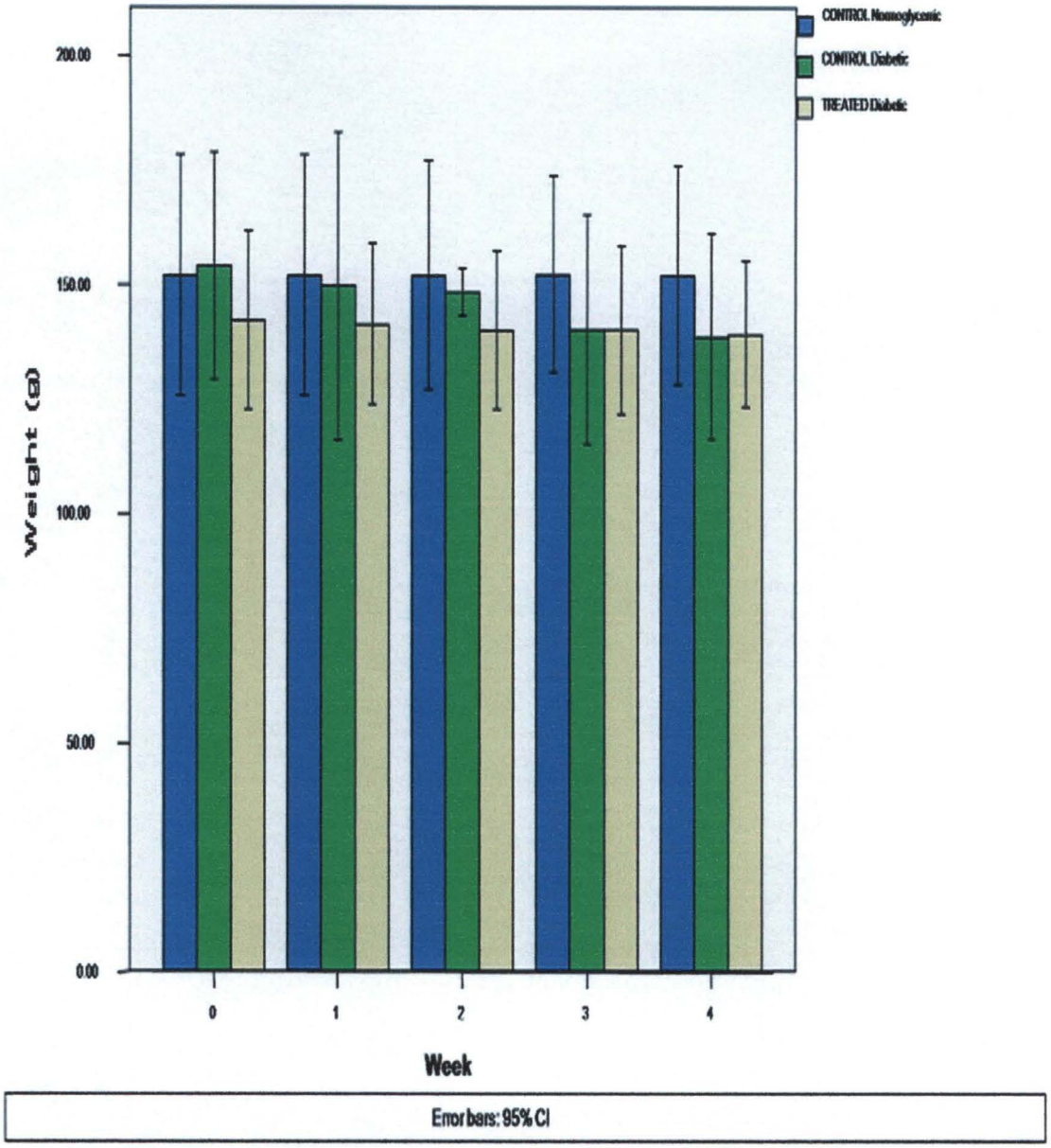


FIG 4.50: Effect of ethylacetate/ethanol fraction (300mg/kg b.wt) of leaf extract of *A. occidentale* on weight of diabetic rats

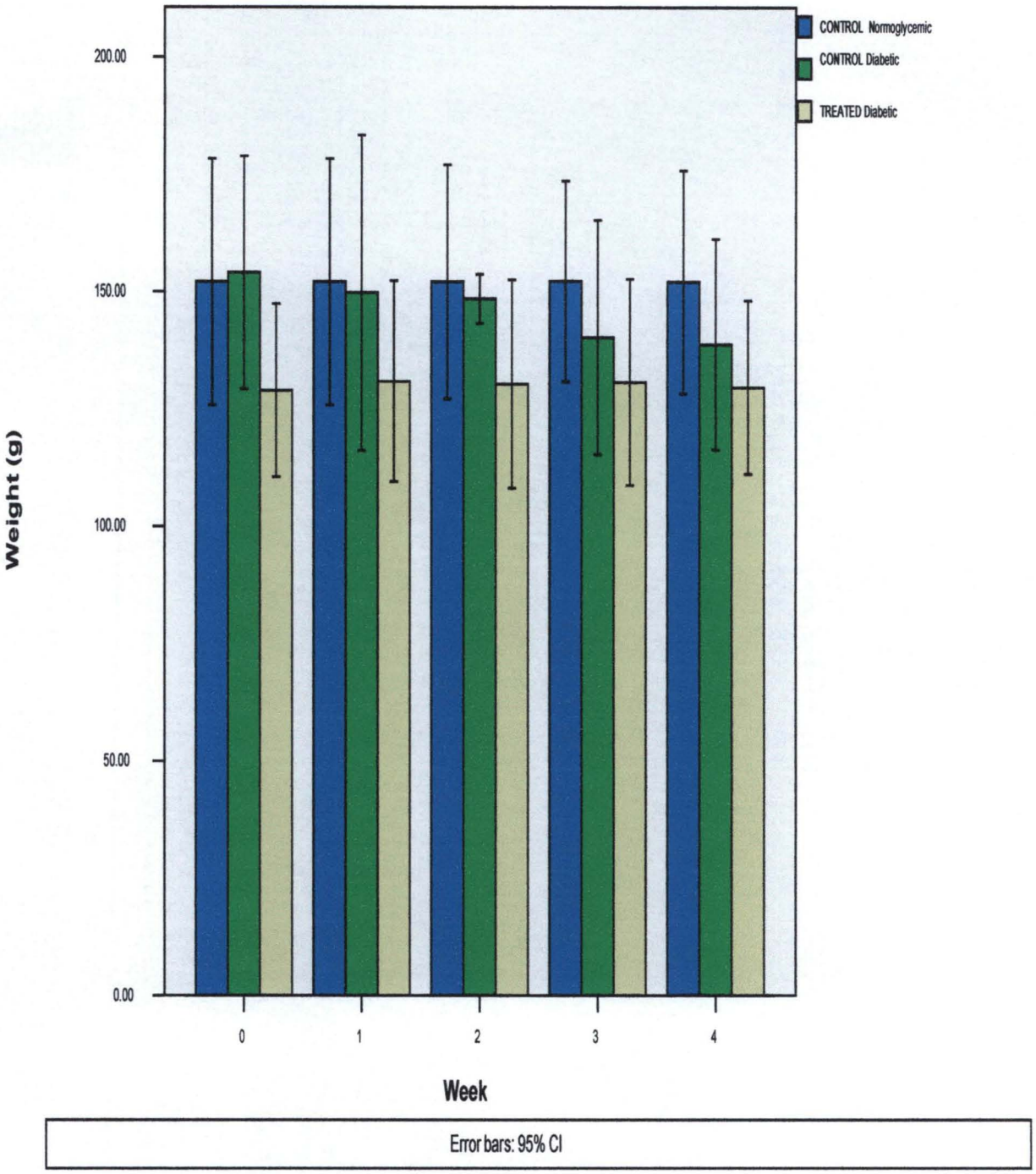


FIG 4.51:Effect of ethanol fraction(300mg/kg b.wt) of leaf extract of *A. occidentale* on weight of diabetic rats.

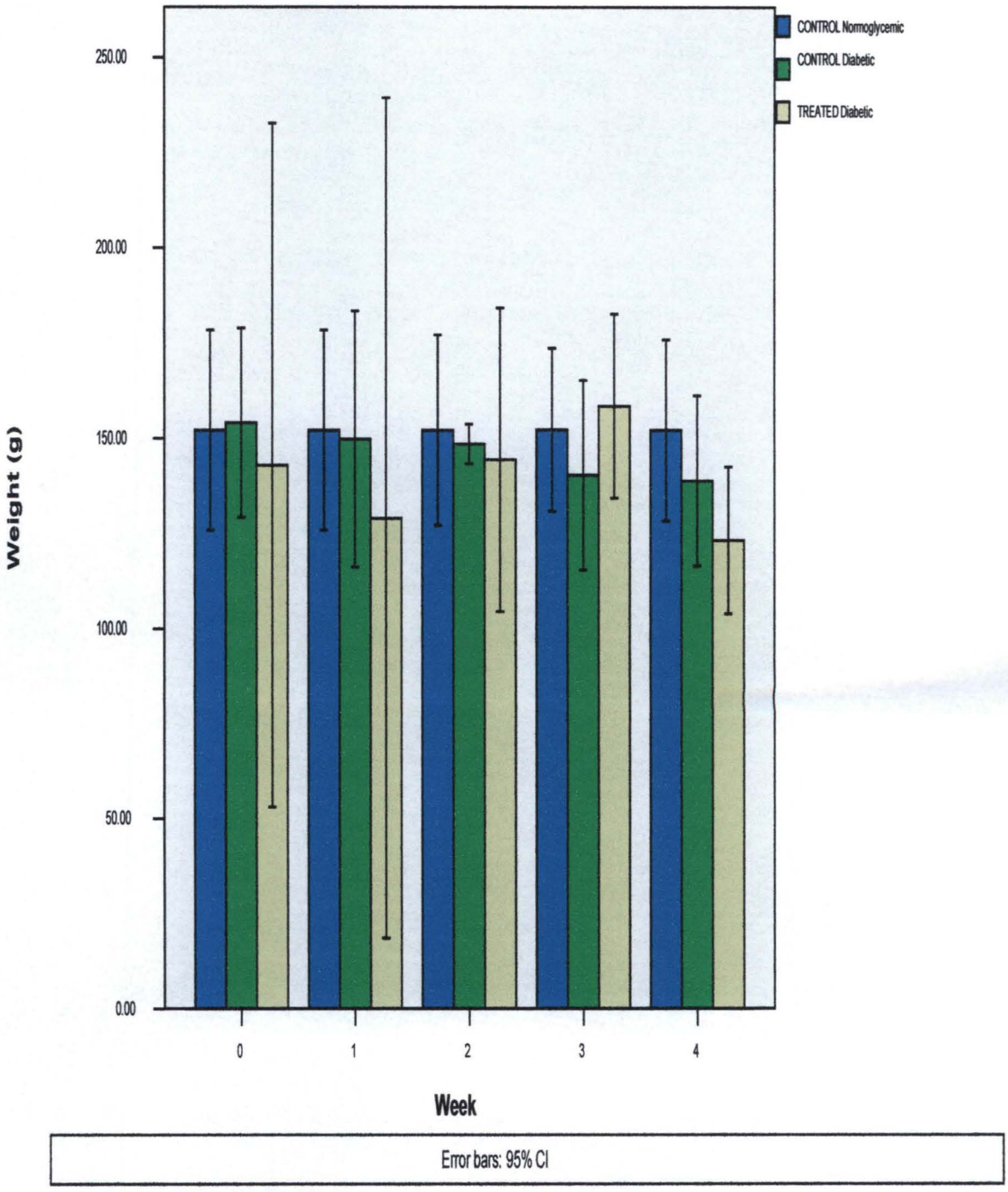


FIG 4.52:Effect of Hexane Fraction (200mg/kgbwt) of leaf extract of *A.occidentale* on the weight of rats.

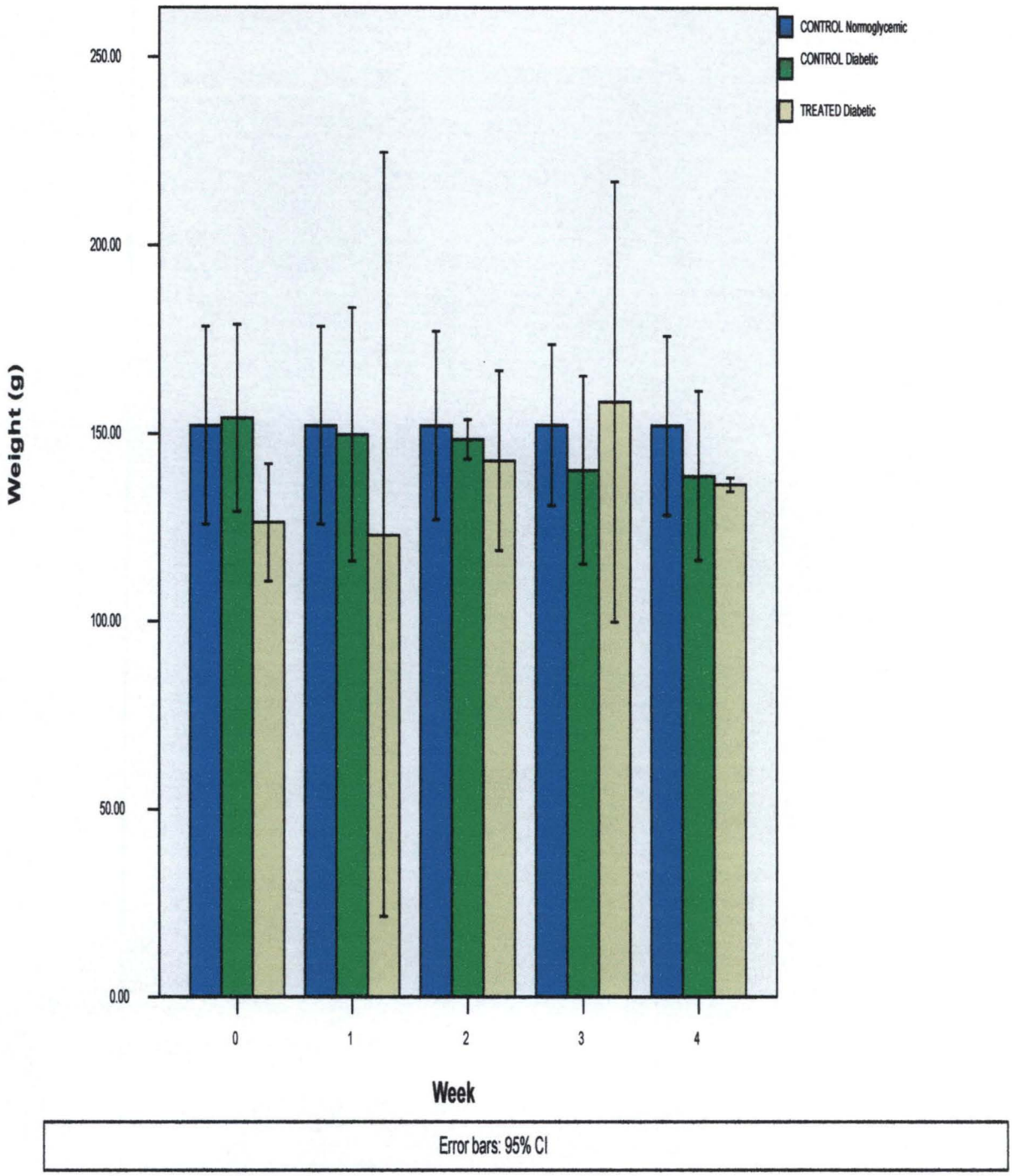


FIG 4.53: Effect of ethylacetate fraction (200mg/kg b.wt) of leaf extract of *A. occidentale* on weight of diabetic rats.

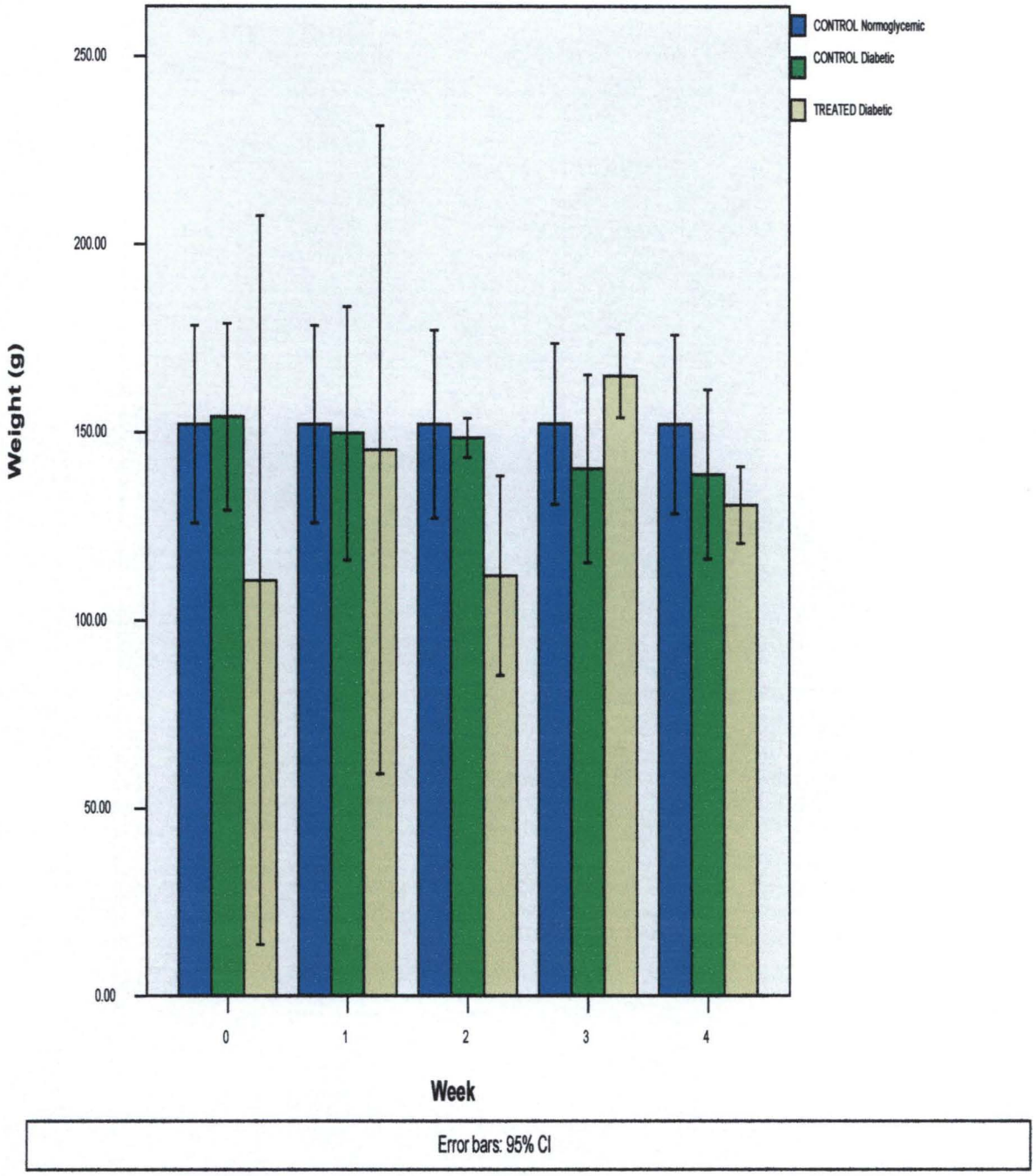


FIG 4.54:Effect of ethylacetate/ethanol fraction [200mg/kg b.wt] of leaf extract of *A. occidentale* on weight of diabetic rats.

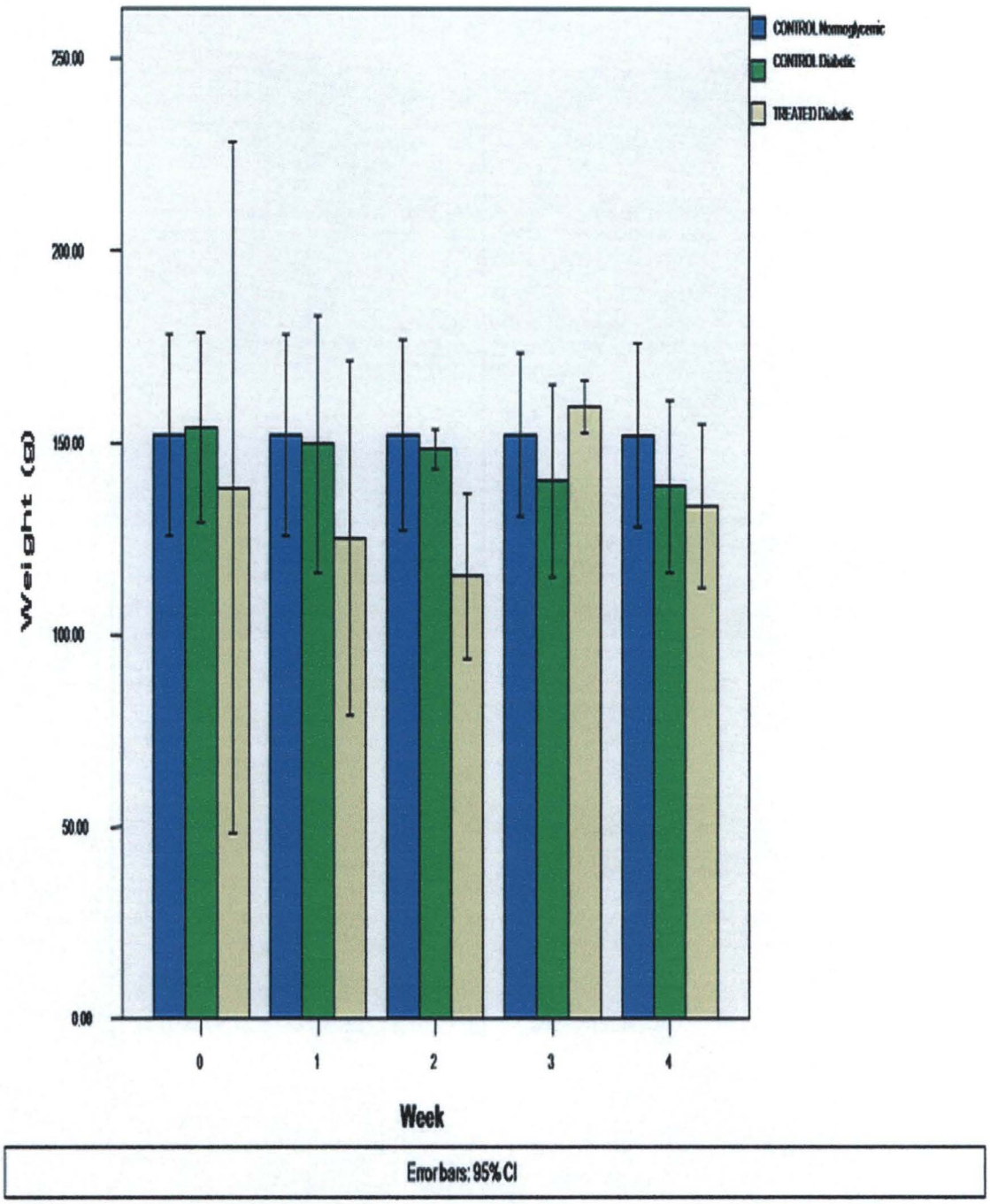


FIG 4.55: Effect of ethanol fraction (200mg/kg b.wt) of leaf extract of *A.occidentale* on weight of diabetic rats

4.26 Effect of Stepwise Fractions of stem bark extract of *Anacardium occidentale* on weight of Diabetic Rats.

300mg/kg bwt

The results of hypoglycemic properties of stepwise fractions (300mg/kg bwt) of stem bark extract of *A occidentale* are shown in Figs 4.56-4.59. The hexane and ethylacetate fractions non significantly ($P>0.05$) decreased the weight of rats compared with control (Figs 4.56 and 4.57). However, the ethylacetate/ethanol and ethanol fractions significantly ($P<0.05$) lowered the weight of rats compared with control as shown in Figs 4.58 and 4.59 respectively.

200mg/kg bwt

The results of the hypoglycemic properties of stepwise fractions stem bark extract of *A.occidentale* are presented in Figs 4.60-4.63. The hexane and ethylacetate/ethanol fractions non significantly ($P>0.05$) decreased the weight of rats when compared with control as shown in Figs 4.60 and 4.62 respectively.

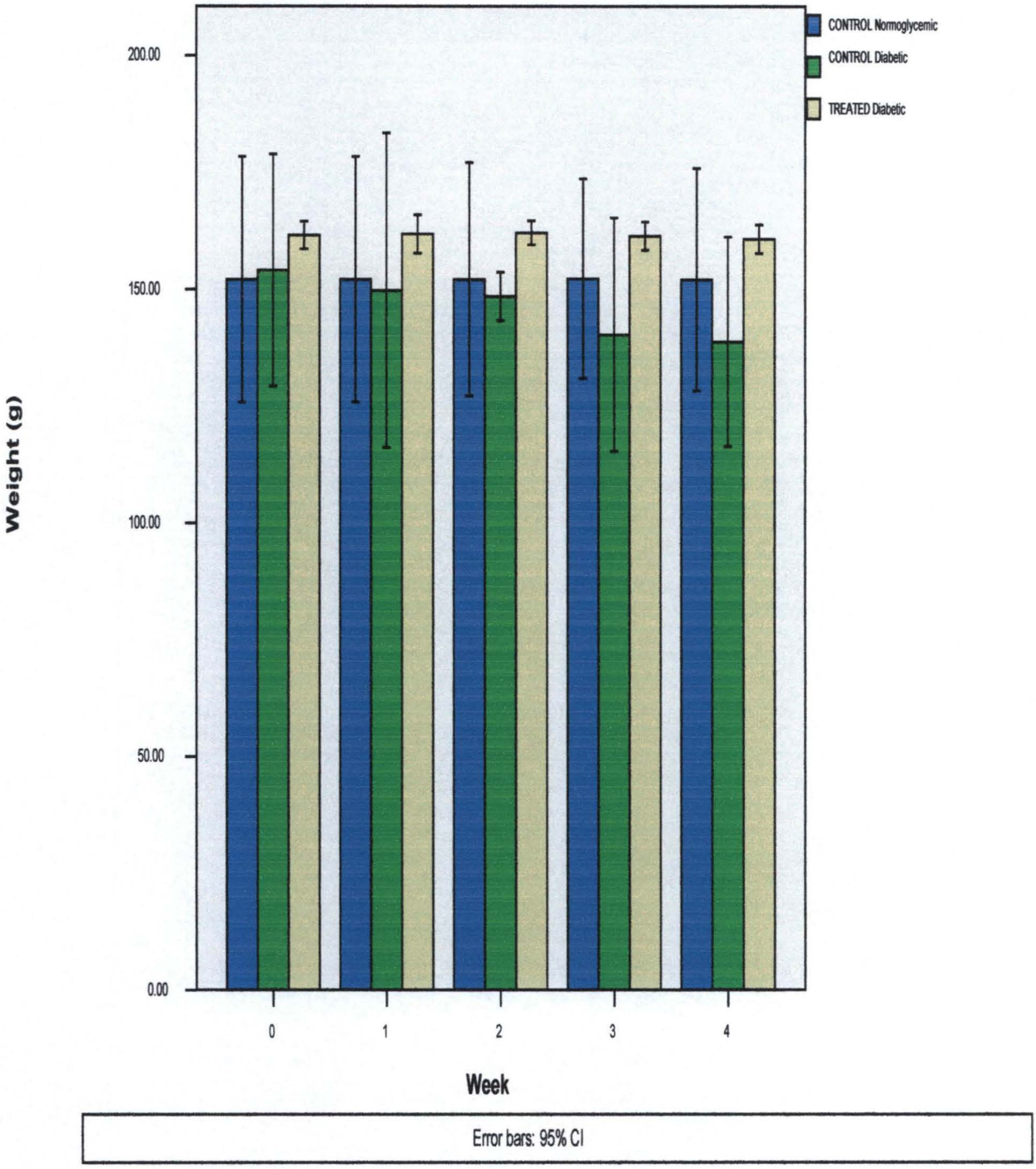


FIG 4.56:Effect of Hexane fraction(300mg/kg b. wt) of stem bark extract of *A. occidentale* on weight of diabetic rats.

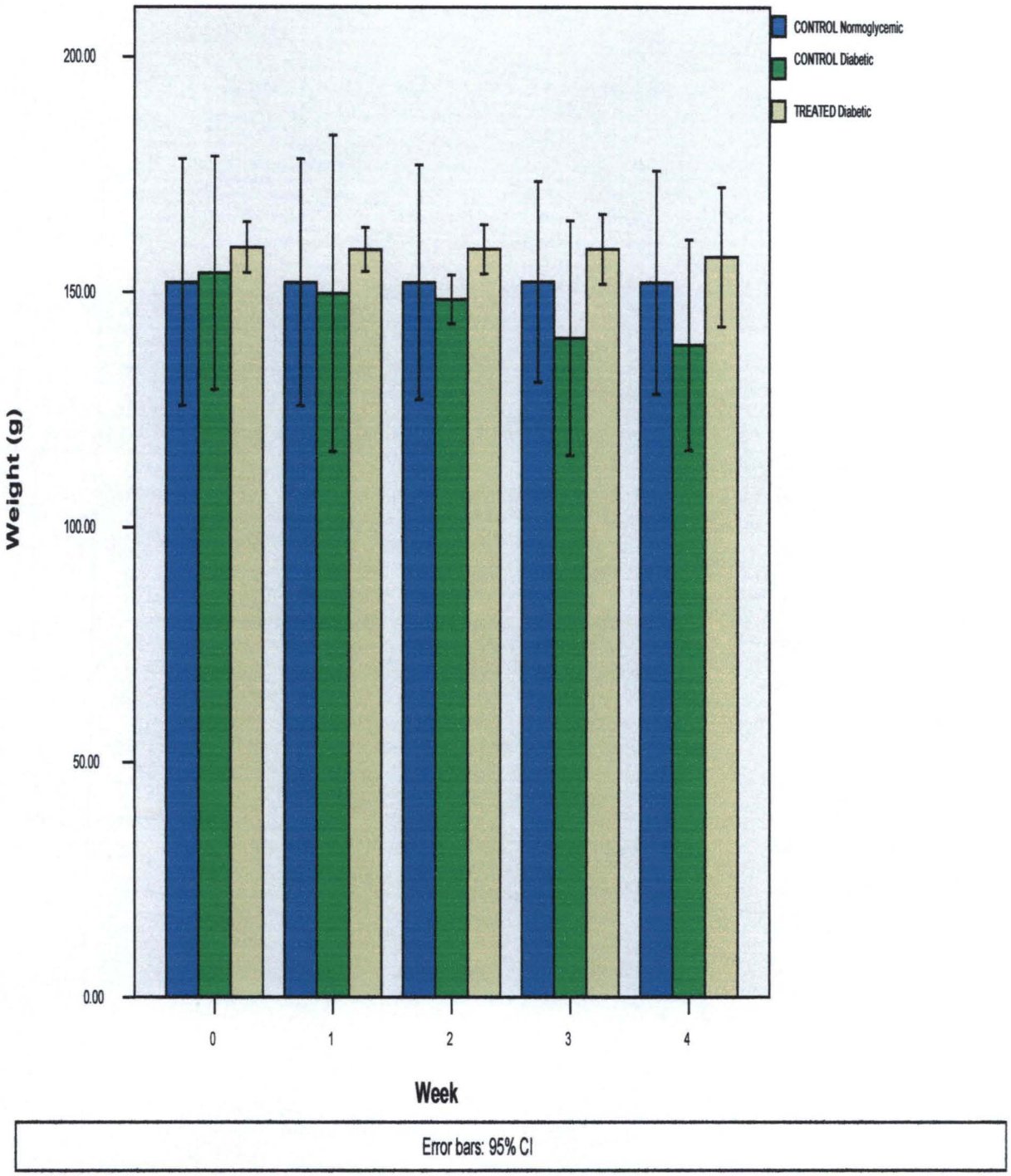


FIG 4.57:Effect of ethlyacetate fraction(300mg/kg b.wt) of stem bark extract of *A. occidentale* on weight of diabetic rats.

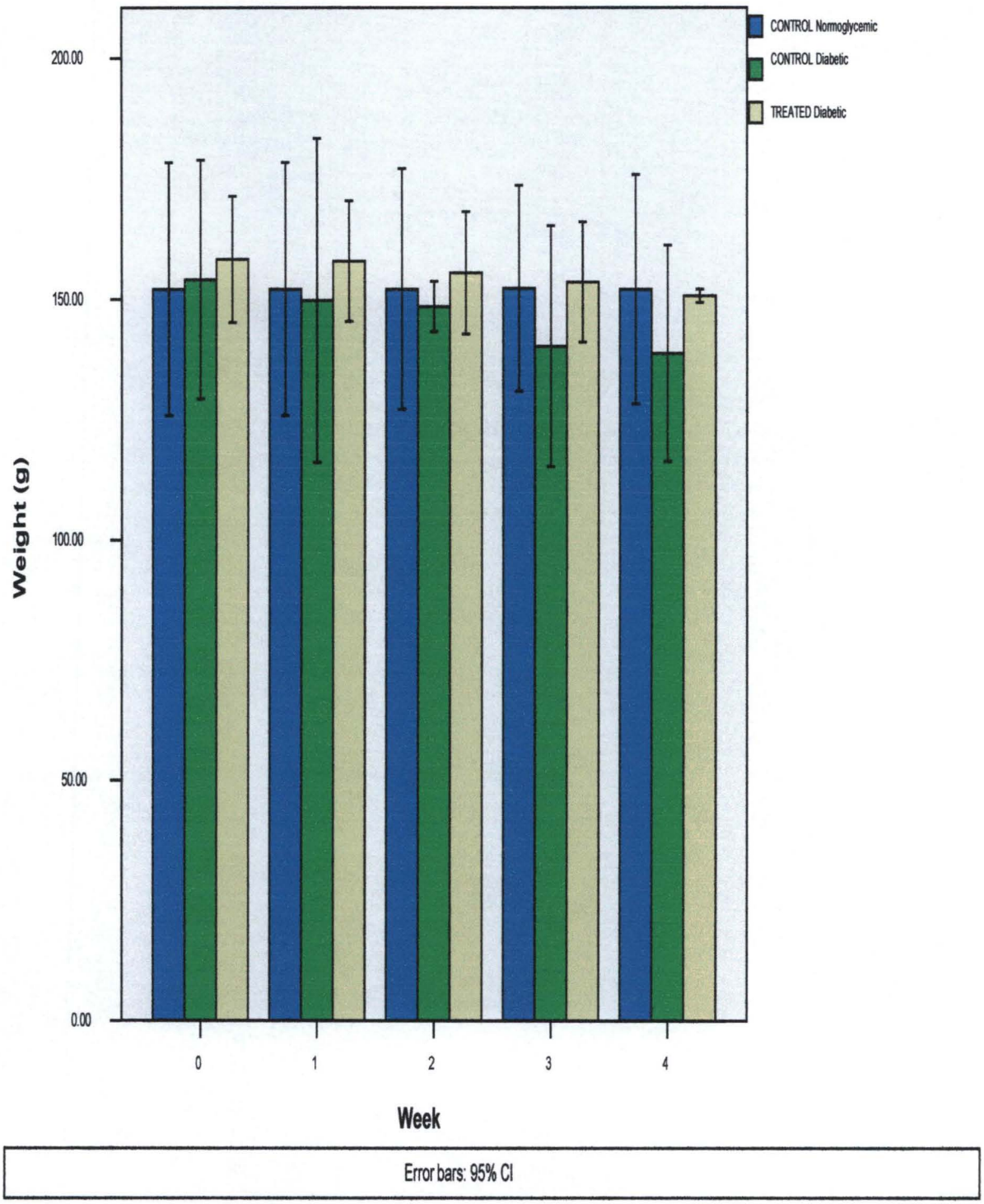


FIG.4.58. Effect of ethylacetate/ethanol fraction(300mg/kg b.wt) of stem bark extract of *A. occidentale* on weight of diabetic rats.

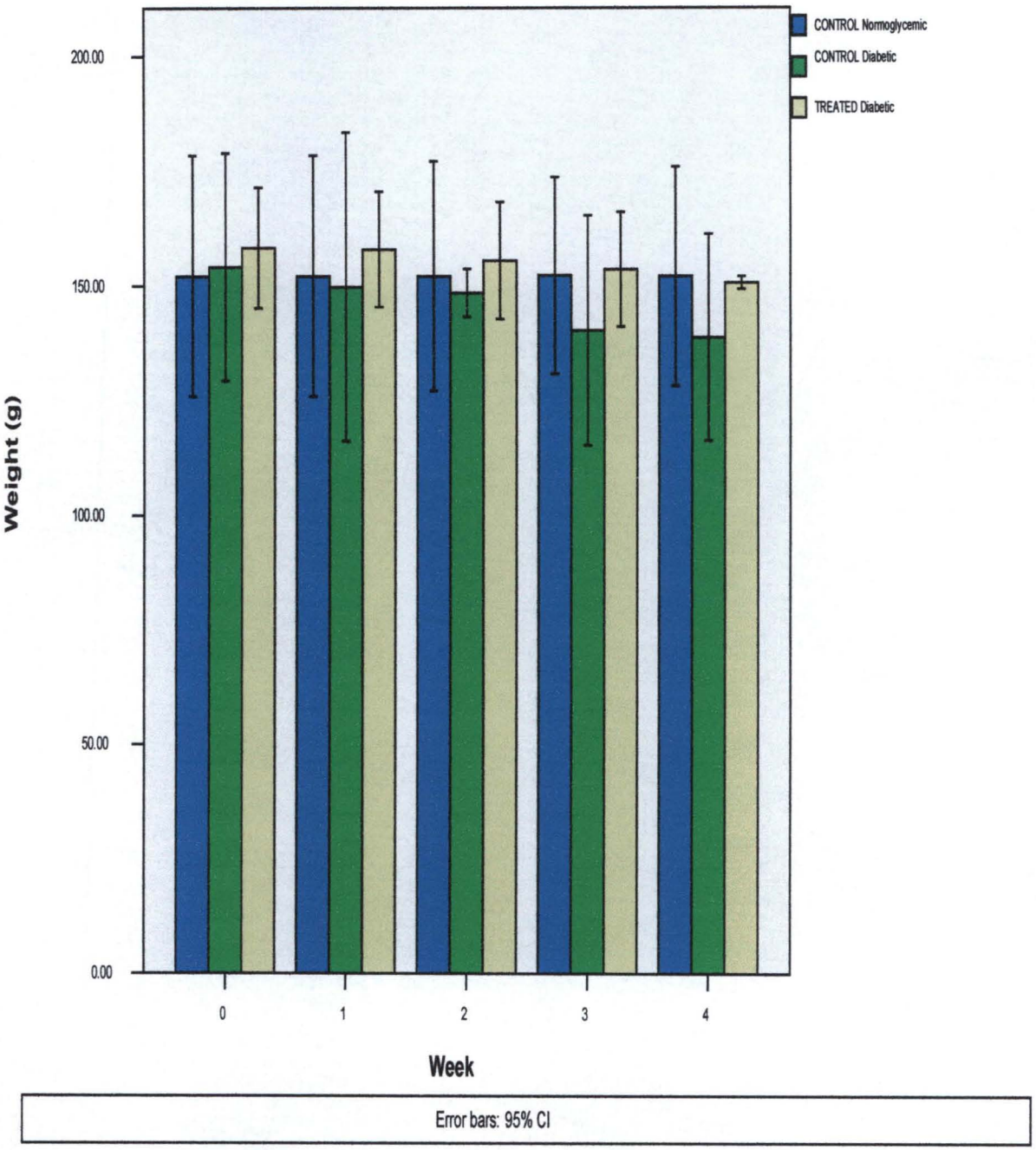


FIG4.59: Effect of ethanol fraction(300mg/kg b.wt) of stem bark extract of *A. occidentale* on weight of diabetic rats.

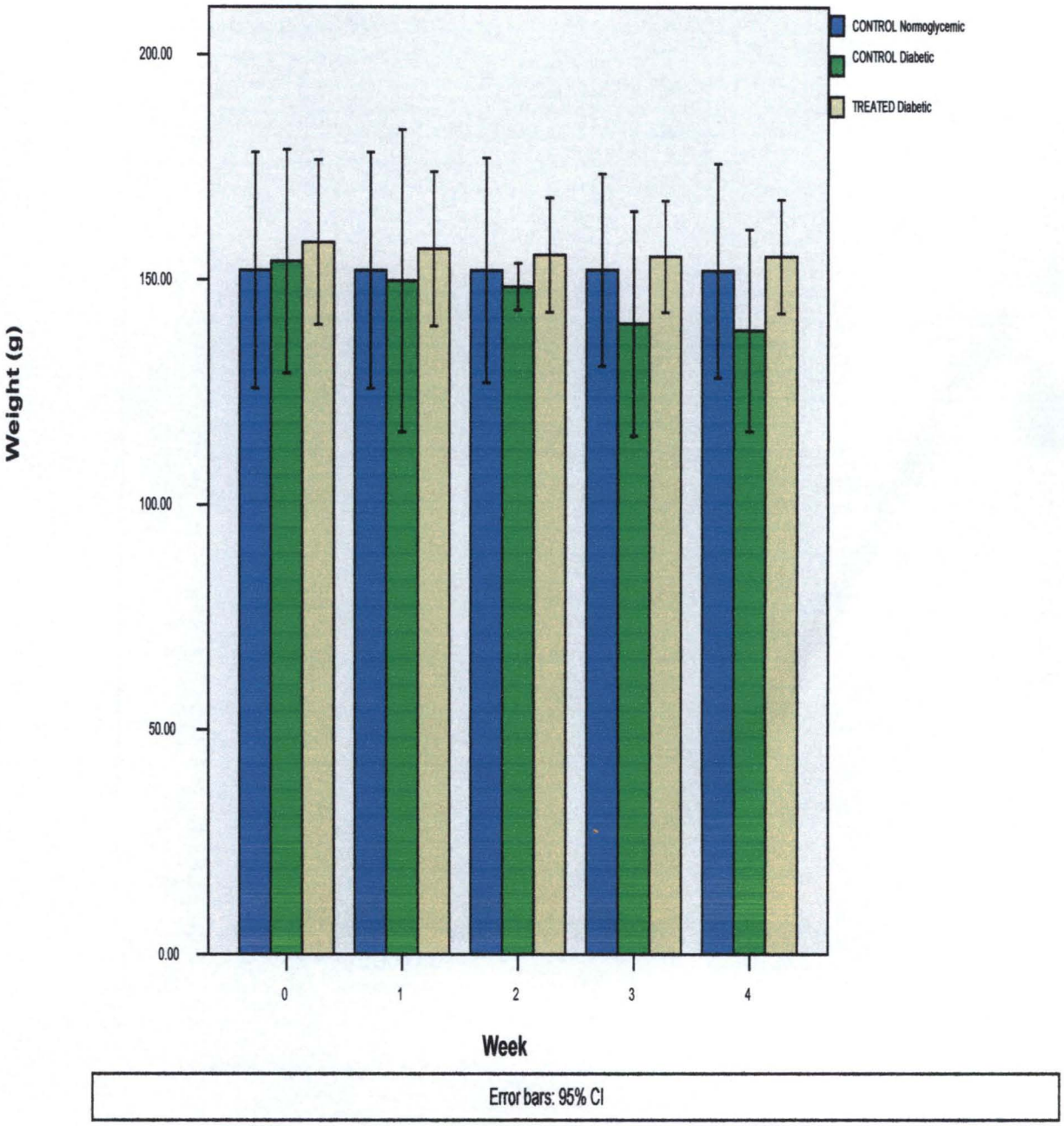


FIG 4.60:Effect of Hexane fraction(200mg/kg .b.wt) of stem bark extract of *A. occidentale* on weight of diabetic rats.

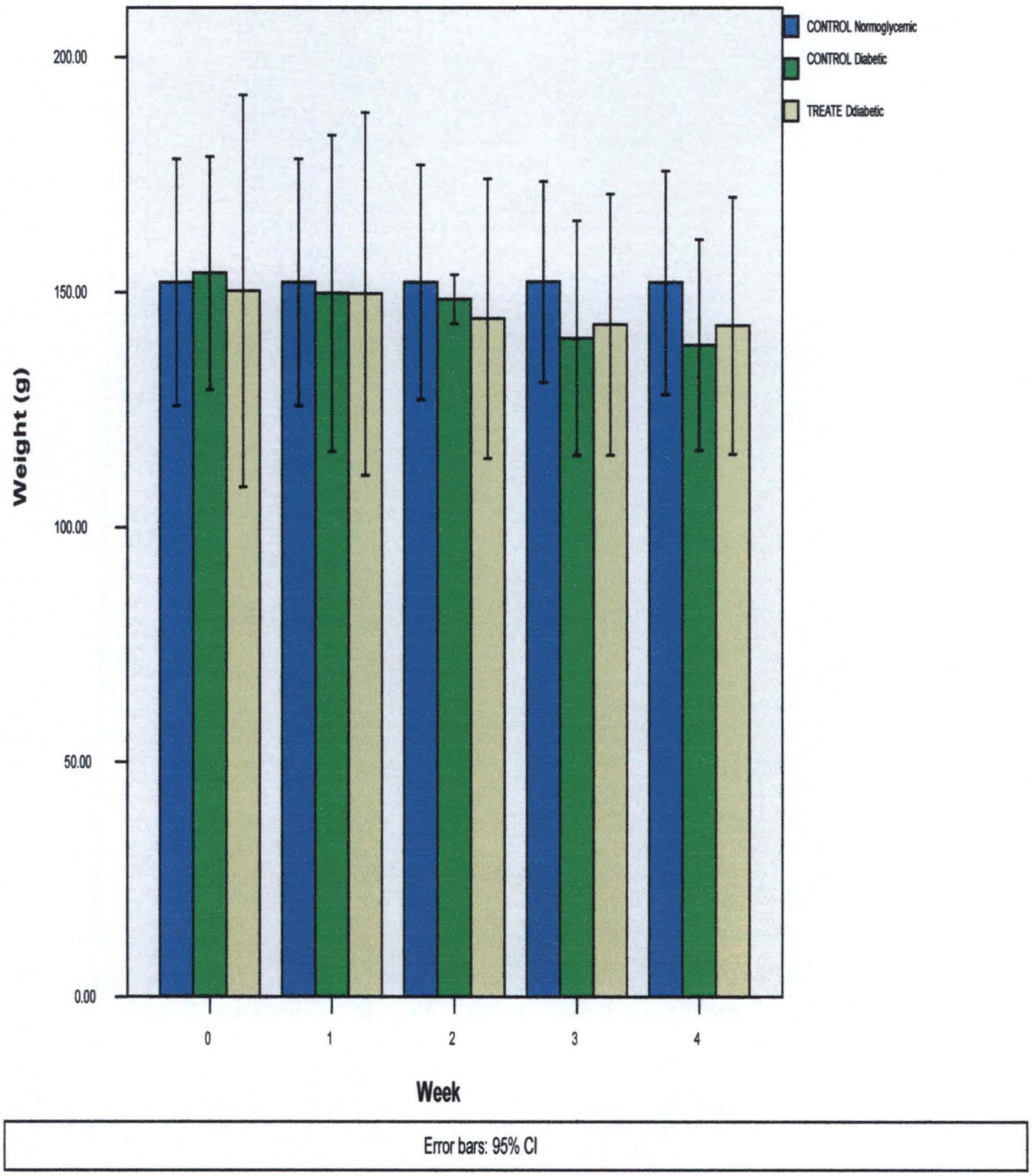


FIG 4.61:Effect of ethylacetate fraction(200mg/kg b. wt) stem bark extract of *A. occidentale* on weight of diabetic rats.

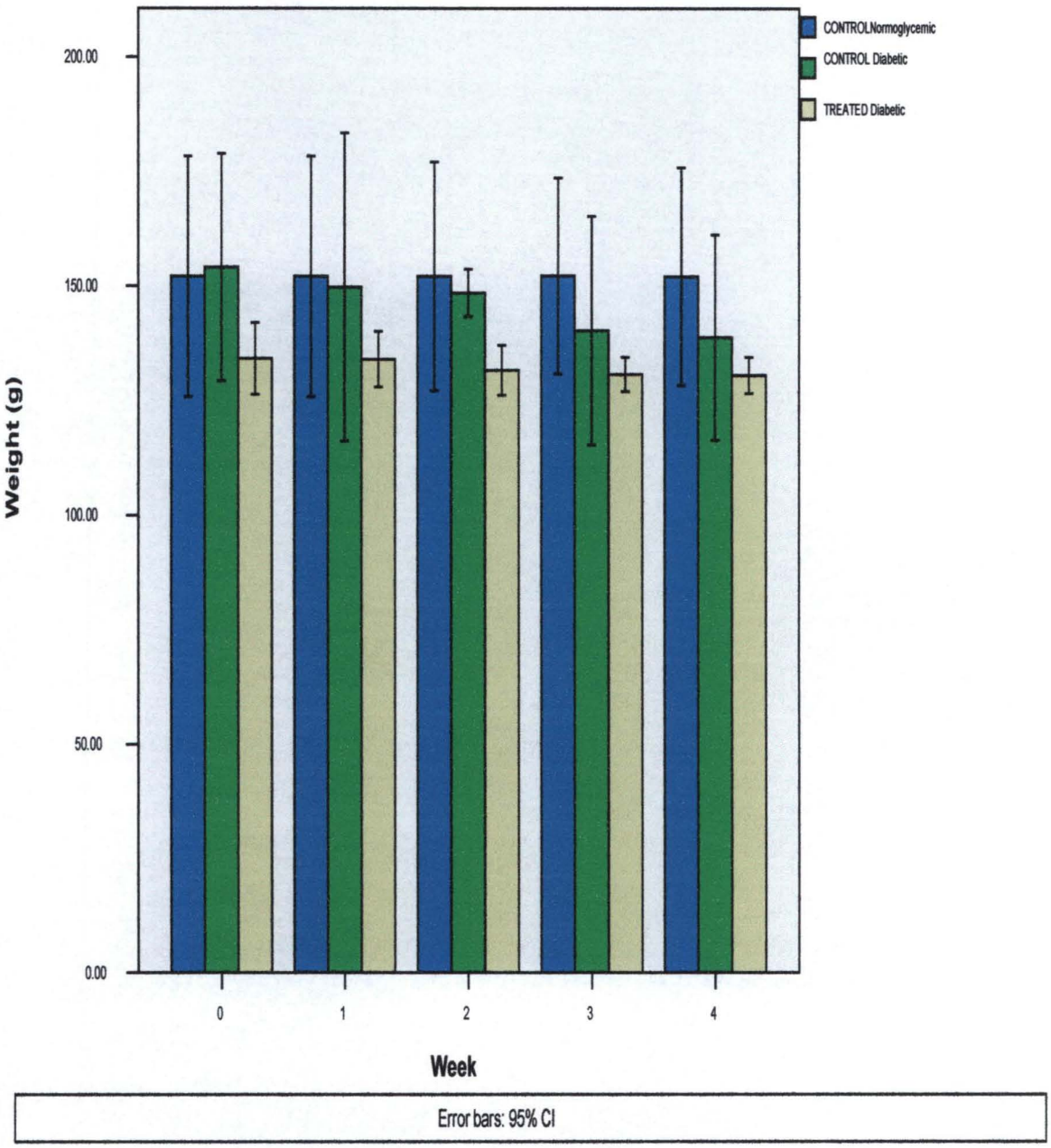


FIG 4.62:Effect of ethylacetate fraction [200mg/kg b.wt] of stem bark extract of *A.occidentale* on weight of diabetic rats.

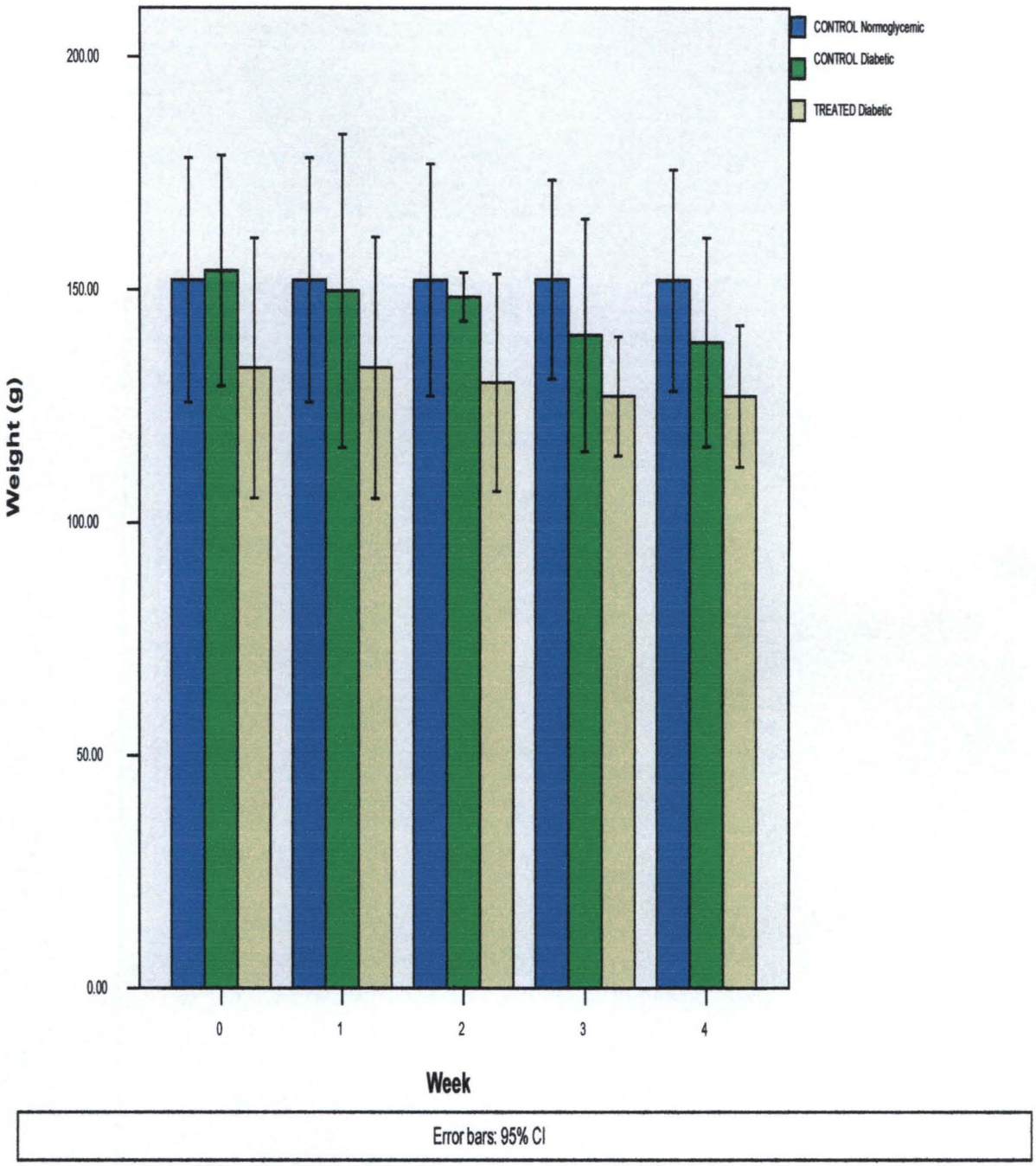


FIG 4.63: Effect of ethanol fraction (200mg/kg b.wt) of stem bark extract of *A. occidentale* on weight of diabetic rats.

4.27 Effect of Ethanolic extract of *Anacardium occidentale* leaf on Organ weights (liver, kidney, spleen, and pancreas) of Rats

The effects of different doses of ethanolic extract of *A.occidentale* on organ weight of rats are as shown in Fig 4.64. There was significant ($P<0.05$) decrease in the weight of liver for groups I (2000mg/kg bwt) and III (6000mg/kg bwt) rats respectively compared with control. The weight of the kidney decreased significantly ($P<0.05$) for group I rats only, while the weight increased for groups II (4000mg/kg bwt) and III rats respectively. The weight of the pancreas increased significantly ($P<0.05$) for all the groups while the weight of spleen remain unchanged when compared with control.

4.28 Comparative Hypoglycemic effect of Column effluents of ethanolic fraction of leaf extract of *Anacardium occidentale* and Metformin on Serum Glucose Concentration of Diabetic Rats.

The results of comparative hypoglycemic effect of column effluents of ethanolic fraction of leaf extract of *A.occidentale* are presented in Fig 4.65. Ethylacetate effluent (200mg/kg b.wt) showed same glucose lowering effect (54.1%) like metformin (500mg/kg b.wt) while ethylacetate effluent (300mg/kg b.wt) had a higher activity (59.6%) than metformin. The ethanol effluent had no activity.

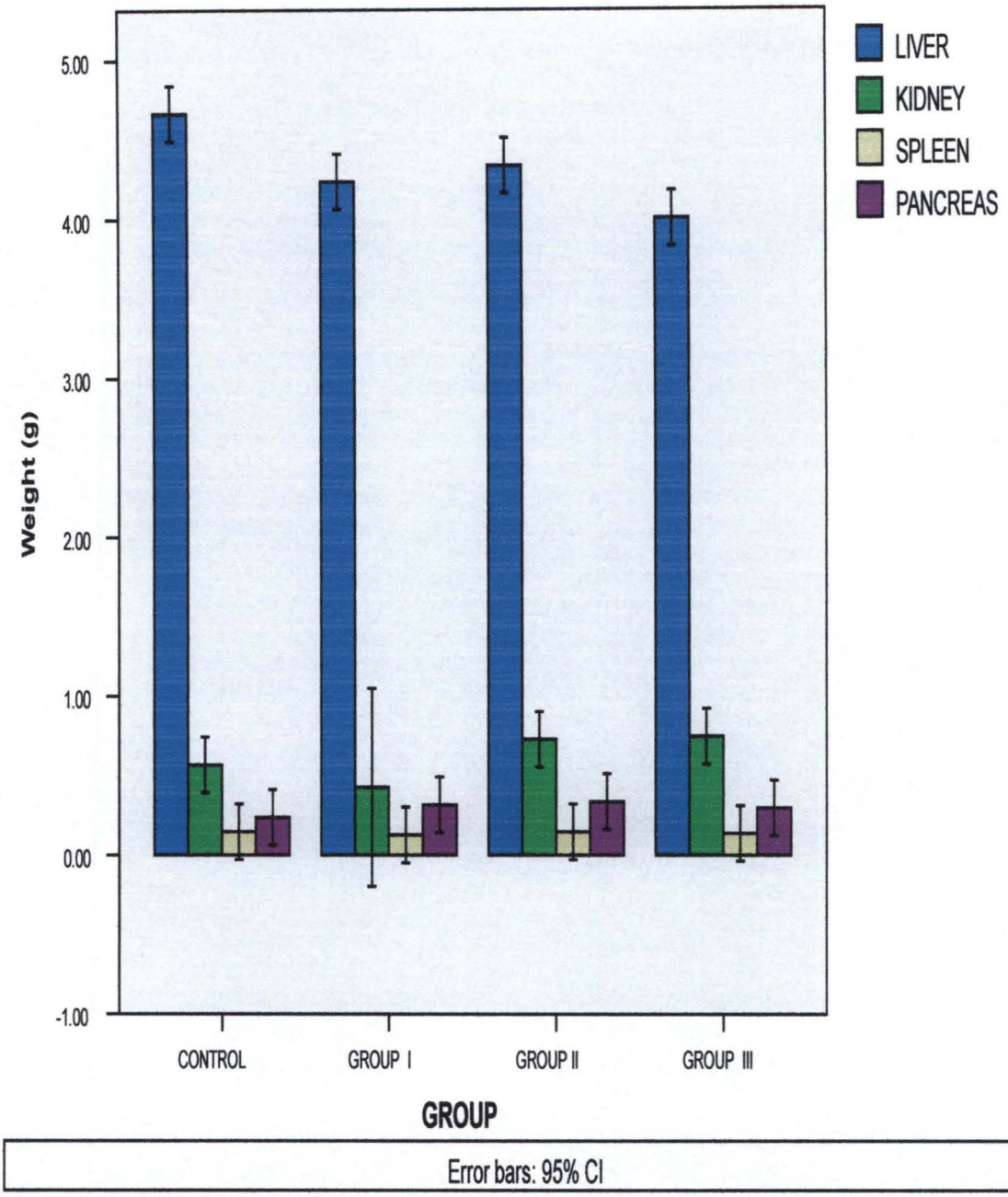


FIG 4.64: Effect of Ethanolic Extract of *Anacardium occidentale* leaf on Organ Weight of rats.

Group I=rats fed 2000mg of extract

Group II=rats fed 4000mg of extract

Group III=rats fed 6000mg of extract

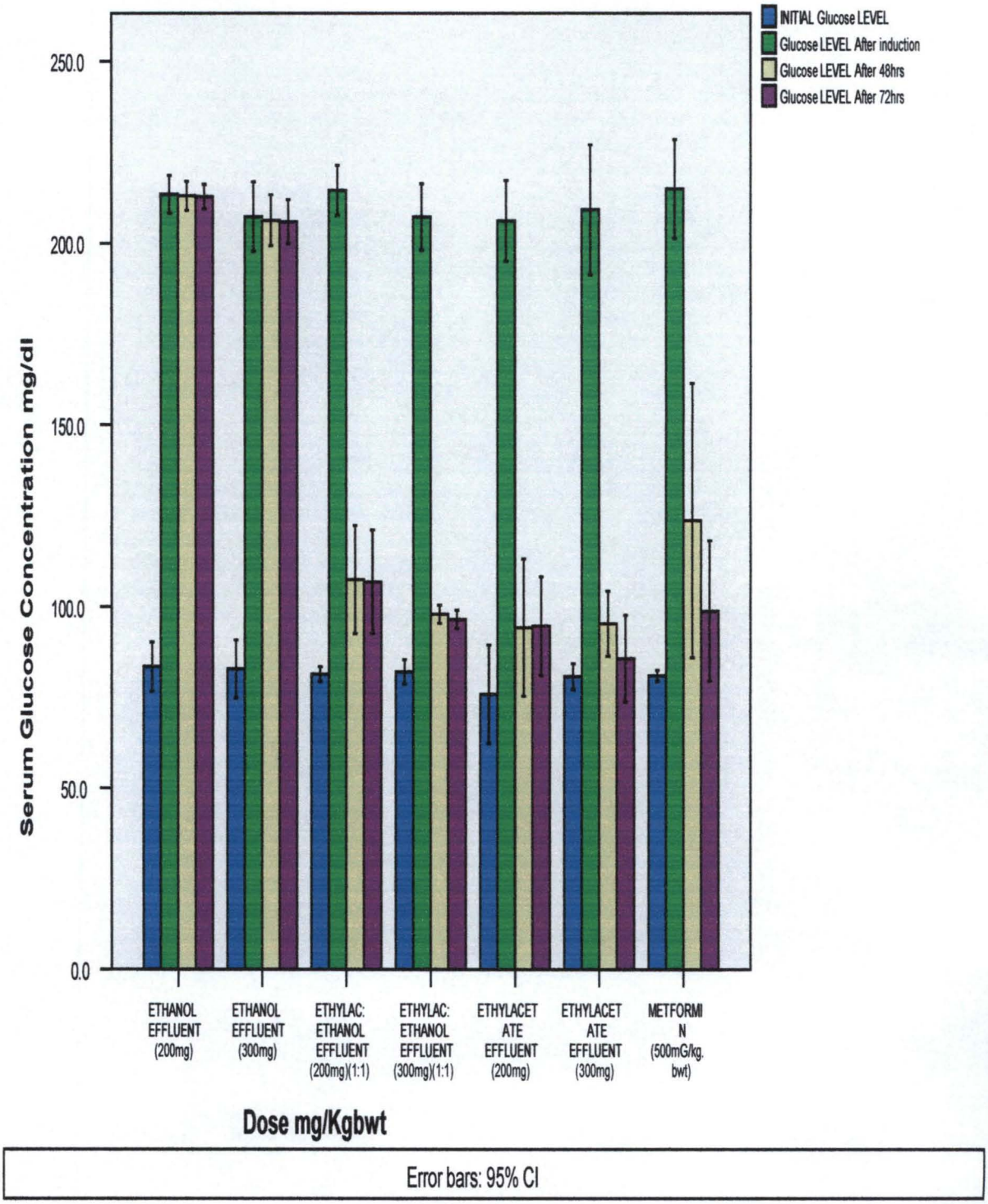
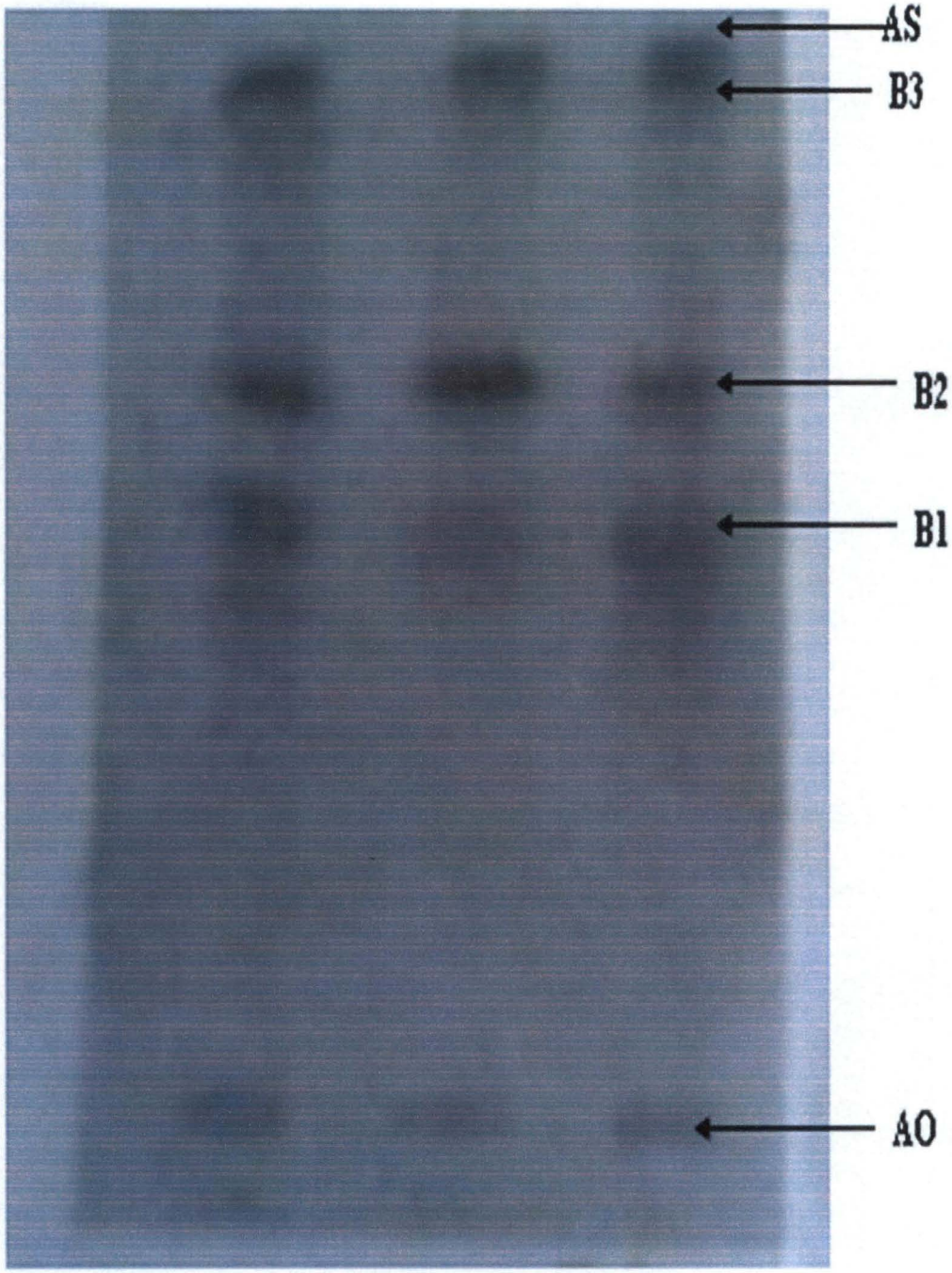


FIG 4.65: Comparative Effect of Metformin and different column effluents of ethanolic fraction of leaf extract of *A.occidentale* on serum glucose concentration

4.29 Thin Layer Chromatography of Ethanolic extract of *Anacardium occidentale* leaf.

The Thin layer chromatogram of the ethanolic extract of *A. occidentale* leaf is depicted in Fig 4.66. The chromatogram showed 3 bands B1, B2 and B3. The RF values for B1, B2 and B3 are 0.50, 0.65 and 0.95 respectively.



AS = Solvent front, B = Band 1(0.50), B2(0.65),B3(0.95),AO = Origin

Fig 4.66: Thin Layer Chromatogram of ethanolic extract of *A.occidentale* leaf using Hexane/Ethylacetate/Ethanol (2:7:1) solvent system.

4.30 Antibacterial Activity of Stepwise fractions of *A.occidentale* leaf.

The results of the antibacterial activity of stepwise fractions of the *A.occidentale* leaf extract are shown in Plates I-II. Ethylacetate/ethanol fractions showed zones of inhibition on *Kliebsiella* (Plate I), while the hexane fraction inhibited the growth of *Salmonella typhi* (Plate II). The ethylacetate fraction inhibited *Staphylococcus aureus* as shown in Plate III.

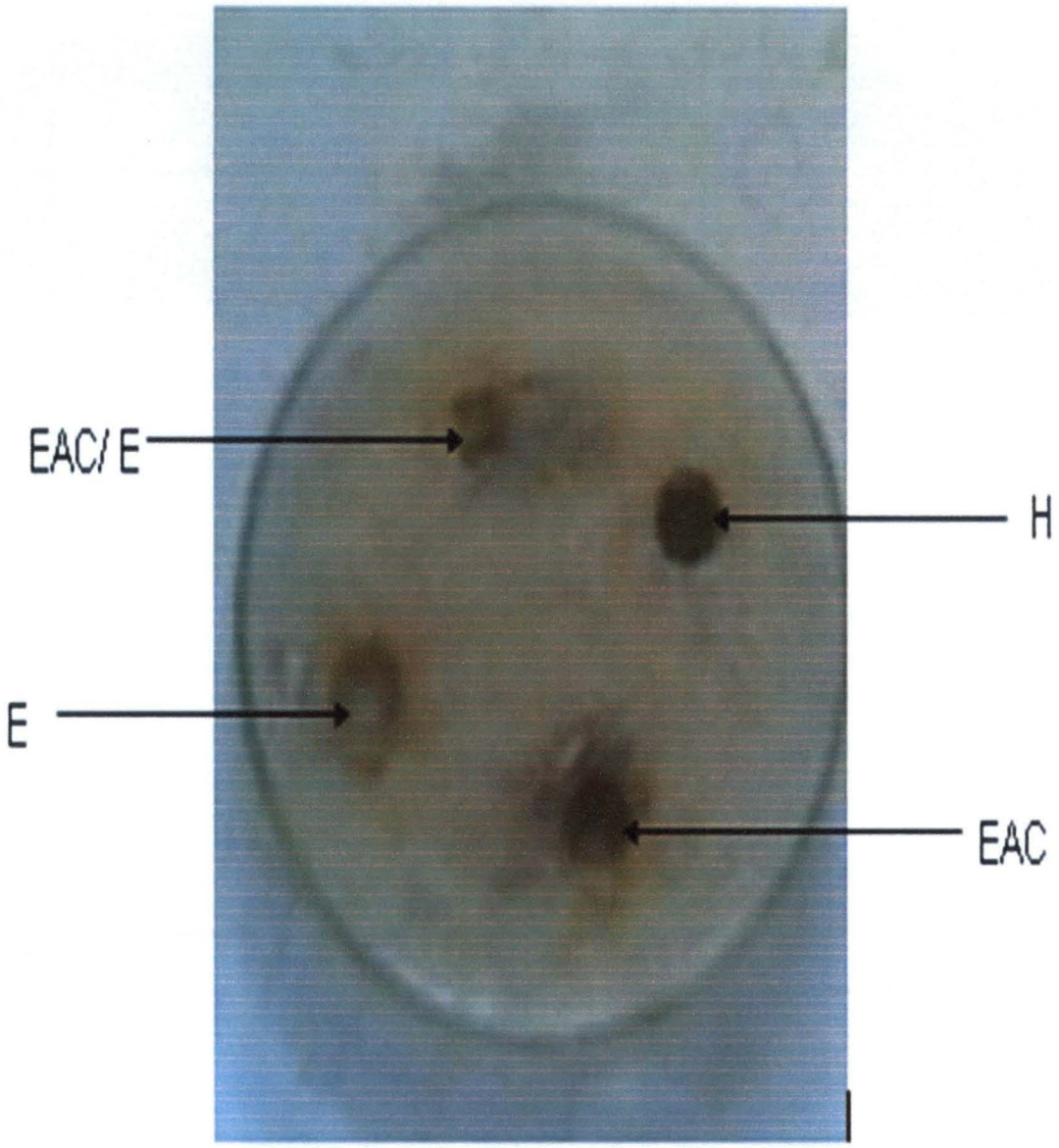


Plate I: Effect of stepwise fractions of *A. occidentale* leaf extract on *klebsiella pneumonae*. Only ethylacetate/ethanol and ethanol inhibits *Klebsiella*.

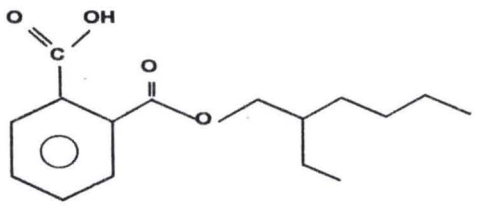
(**Key**: H-Hexane, EAC-ethylacetate, EAC/E-ethylacetate/ethanol, E-ethanol)

4.32 Spectral Analyses of Column Effluents of Ethanolic Fraction of *A.occidentale* leaf extract.

Gas Chromatography-Mass Spectroscopy and Infra Red.

The spectral analyses of the most active hypoglycemic effluent (ethyl acetate) of *A.occidentale* leaf are presented in Figs 4.67 and 4.68. The GC-MS spectral technique indicates the presence of an ESTER as the likely active principle (Fig 4.67). The ester found in the fraction had the following structure based on the National Institute of Science and Technology library search.

1. 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester-MEHP
2. $C_{16}O_4$
3. M.wt = 256.1688
4. Cas No = 4376-20-9

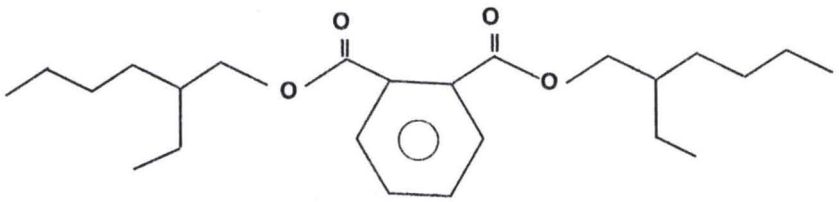


Formula	$C_{16}H_{22}O_4$
Molecular wt.	278
CAS No.	4376-20-9

1,2 – Benzenedicarboxylic acid, mono(2-ethyl)ester

Another likely ester found is:

1. Bis (2-ethylhexyl) phthalate – DEHP
2. $C_{24}H_{38}O_4$
3. M.wt = 390.5561
4. Cas No 117-81-7



Bis (2-ethylhexyl) phthalate

Formula	$C_{24}H_{38}O_4$
Molecular wt.	390
CAS No.	117-81-7

The IR spectra of the same ethyl acetate effluent revealed that the peaks between 1,700 and 1,750 are ester peaks (Fig 4.68). It further confirms that the most active effluent is an ester.

CHAPTER FIVE

DISCUSSION

5.1 DISCUSSION

5.1.1 Crude Extract Yields

The crude extract yields (Table 4.1) of the plants studied were within the range obtained in some literature and are quite appreciable (Tona *etal*, 1999). The highest percentage crude extract yield was obtained from aqueous extract of *Anacardium* stem bark (80%) followed by that obtained with the leaves(70%).This signifies the differences in organs used (Kirby, 1997; Wilcox and Bodekar, 2000). It also suggests that the *Anacardium* leaves and stem bark contains more extractable active principles than that of other plants studied.It was reported that anacardium leaves are extensively used in the Cameroon and other African countries for the treatment and management of diabetes and hypertension (Tedong *etal*, 2006; Sekong *etal*, 2001).

5.1.2 Safe Dose and LD50 of Plants

Two of the six different aqueous extracts of the plants studied (Table 4.2) namely, *Blighia sapida* and *Anacardium occidentale* leaves had low safety margin (<500mg/kg Bw) and the rest had high safety margin (>500mg/kg Bw).This is an indication that the two plants mentioned above may be toxic (Table 4.3). The non-toxic nature of the other plants might be the reason why they are widely consumed as vegetables. e.g *Zyzzipus spinachristi* and *Moringa oleifera* (Benoit-vical, 2003).

5.1.3 Phytochemical Screening of Crude Extracts.

Qualitative phytochemical screening of the extracts (Table 4.7) revealed the presence of tannins, saponins, cardiac glycosides, alkaloids, flavonoids, volatile oils, terpenes, indole alkaloids, anthraquinones, cyanophoric glycosides, phlobatanins and resins. The phytochemical diversity of the plants analysed is indicative of their varied therapeutic potentials. Secondary metabolites generally form the basis for the pharmacological and medicinal effects of the plant species (Haidet, 2003). Tannins have been reported to be the most widespread in the plant kingdom. They are responsible for most behavioural toxicities induced by medicinal plants (Muyibi *etal*,1999). They arise partly as a result of tissue response to injury.Such cellular disruption release polyphenol oxidases and phenolic substrate e.g gallic chlorogenic and caffeic acids . (Irosin,1982). The low incidence of cynaophoric glycosides found in some plants analysed indication that they may be relatively safe to use. A high level of this metabolite in foods and drugs is undesirable because cyanophoric glycosides can be hydrolysed invivo releasing cyanide, a potent inhibitor of the respiratory chain (Gamaniel, 2000).

It has been reported in literature, that the biological activites of alkaloids and flavonoids include hypoglycemia,hypolipidemia, hypoazotemia and hypotension among other biological effects (Oladele *etal*, 1995; Sudhesh *etal*, 2005). The presence of these two metabolites in high concentration in the extract may be responsible for the oral hypoglycemic effects recorded in the present study (Farkes, 1980; Verticheran and Jegadeeson, 2002). Epicatechin, a flavonoid compound is reported to promote regeneration of beta-cells of islet of Langerhans (Gupta, 1994). It is interesting to note that many plants polysaccharides have also been reported to

exhibit hypoglycemic effect (Ling-hua and Pei-Gren, 1993; Marles and Farnsworth, 1995; Perez *etal*, 1998).The screening revealed that balsams are completely absent in all the plants indicating absence of detectable cinnamic acids in the plants(www.knowledgerush.com).

Phytochemicals detected in the plants analysed agreed with other reports in literature on the phytochemical constituents found in other plants e.g El-Sayyad and Ross (1983) and Al-Ghazali *etal* (1987) reported tannins,tropane alkaloids, saponins and flavonoids in *A.nilotica*; Carter (2001) reported saponins , flavonoids and tannins in Z.officinale. Male etal (1977) reported alkaloids, resins in *G.senegalensis*. Evans (2000) reported alkaloids, cardiac glycosides, tannins, saponins and flavonoids in M.balsamina. Oladimeji *etal* (2000) and Pascual etal (2001) detected alkaloids, glycosides, tannins, resins and volatile oils on *L.multiflora*. Dalzie (1986), Taira etal (1999) and Aquaye *etal* (2004) found saponins, flavonoids, morphine alkaloids in *X.aethiopica*. In many cases, phytochemicals serve as the molecules of defence for plants against predation by micro-organisms, insects and herbivores. Some are involved in plant odour (Terpenoids), pigmentation (Tannins and quinines) and flavour (Capsacin). However, several of these metabolites posses medicinal properties (Cowan, 1999).

5.1.4 Effect of Crude Aqueous Plant Extracts.

The effect of crude aqueous plant extracts were investigated in normoglycemic and diabetic rats. For normoglycemic rats, a significant weight loss by the rats was recorded on administration of crude extract of A. occidentale leaf (Fig 4.13) while a fluctuation in weight was observed for the crude extracts of

Moringa oleifera(Fig 4.21) and *Blighia sapida* leaves(Fig 4.30). For diabetic rats, a significant weight increase in weight on treatment was observed with crude extracts of A. occidentale(Fig 4.17) and *Blighia sapida* leaves(Fig 4.9) while a fluctuation in weight was observed for *Moringa oleifera* leaf(Fig 4.25).The loss in weight might be as a result of alterations in the tissue metabolic pathways or variations in the physiological status of the rats. The increase in weight may be linked to an improvement in the uptake of glucose on treatment with extract. Another reason might be due to stimulation of hormones and enzymes associated with growth. This is in agreement with findings by Tedong, 2006 in which an increase in weight was reported on administration of hexane extract of *A. occidentale* leaf to rats.

All the crude aqueous extracts examined had varying hypoglycemic activities.Three of the plants namely: *A. occidentale*, *M. oleifera* and *Blighia sapida* leaves had above 50% serum glucose reduction while the other plants had below 50% serum glucose reduction. For normoglycemic rats, a significant decrease in serum glucose was observed with crude aqueous extracts of *A. occidentale*, *Moringa oleifera* and *Blighia sapida* leaves(Figs 4.14,4.22 and 4.6). The decrease in serum glucose levels might be due to stimulation of the pancreas by the active components of the extract to secrete insulin since there was no induction of diabetes. For diabetic rats, all the extracts examined had significant glucose lowering effects(Figs 4.18, 4.26 and 4.10). However, the aqueous extract of *A. occidentale* leaf had the highest hypoglycemic activity compared with other plant extracts. *Zyzzipus spinachristi, Artemisia herba alba* and *Terminalia glauscens* leaf extracts(Figs 4.29,4.30 and 4.31) had significant glucose reduction ability. In this category, *Terminalia* had the highest hypoglycemic effect compared to the other two

plants.The activities may be due to the active components in the extracts that are capable of mimicking the insulin action since induction of diabetes had destroyed the beta cells of the islets of Langerhans. This is in agreement with other literature reports on same plant. Sokeng *etal*, 2001 reported the glucose lowering ability of hexane extract of *A.occidentale* leaf. M. oleifera leaves were reported to significantly decrease the blood glucose concentration in rats at 20,30,45 and 60 mins compared with control after glucose administration. Major polyphenols in M. oleifera powder such as quercetin glucosides, rutin, kaempferol glycosides and chlorogenic acids were suspected to be responsible (Moussa *etal*,2007). Blighia sapida parts namely: leaves, fruits and seeds are known to contain hypoglycin A and B that inhibits gluconeogenesis(http//emedicine.com/article/81.Chem *etal*,1957). Ahmad *etal* (2008) reported that aqueous and methanolic extract of *Zyzzipus spinachristi* had 53% inhibitory effect on α- glucosidase . The extract was also reported to alter some enzymes involved in carbohydrate metabolism e.g liver phosphorylase and glucose 6 phosphatase. It was reported that *Artemisia herba alba* posses some hypoglycemic principles that have been used in many countries of Middle East and Turkey as herbal medicine (Moussa, 1985; Al- Shammaony *etal*,1994, Maniff *etal*, 1995; Brachmachari and Augusti, 1962). It was also reported that rabbits treated with aqueous extract of *Artemisia herba-alba* (85mg/kg bw) showed antidiabetic effect (Wadood *etal*,1992). It was reported in literature that the aqueous extract of *Terminalia glauscens* effectively brought about the restoration of diabetic indicators such as elevated blood glucose and liver triglyceride concentrations to the normal values in the treated diabetic rats(Onoagbe *etal*, 1999). It was also reported that methanol extract of *T. glauscens* (300mg/kg bw) effectively lowered blood glucose in

streptozotocin induced rats (Njomen *etal*,2009).Burcelain *etal* (1995) reported that hypoglycemic action of the plant extracts in diabetic rats may be possible through insulinomimictic action or stimulation of glucose uptake by peripheral tissues, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles.

The triglyceride and protein levels were also investigated . For normoglycemic rats, there was a significant increase in triglyceride(Fig 4.15) and protein (Fig 4.16) levels on administration of aqueous extract of *A.occidentale* leaf while a fluctuation in the triglyceride and protein levels(Figs 4.7and 4.8) were observed for *Blighia sapida* extract. However, the *Moringa oleifera* leaf extract had no effect on the protein level (Fig 4.23). For diabetic rats, a significant decrease in the triglyceride profile was observed on administration of all the extracts (Figs 4.19,4.27 and 4.11). However, the protein levels for all the extracts fluctuated (Figs 4.20,4.28 and 4.12). On the contrary, it was reported by Jaiswal *etal*, (2009) that the oral administration of *Moringa oleifera* at different doses lead to increased total protein after 21 days of treatment. The increase in triglyceride and protein levels might be due to initial mobilization of the triglyceride and protein into the bloodstream of rats(Jaiswal *etal*, 2009). The fluctuation in triglyceride and protein levels might be due to alterations in the metabolic activities of the rats. However, the decrease in triglyceride level may be as a result of inhibition of enzymes responsible for triglyceride synthesis e.g phosphatidic acid phosphatase and acyltransferases (Tedong, 2007).

5.1.5 Effect of Stepwise Fractions of *A.occidentale* extract in rats..

a. Fractions of the leaf extract.

The ethanolic fraction of the *Anacardium* leaf extract (Fig 4.35) had the highest percentage glucose reduction at 200mg/kg bw. However, at 300mg/kg bw, there was reduced activity.The mode of action of the fractions may be similar to that of drugs that exhibit low activity at higher doses. Another reason might be due to variations or changes in metabolic status of the rats.This finding is in agreement with studies on *Cissampelos muconata* by Tanko *etal* (2000), Chandrika *etal*(2006) and Ogbadoyi *etal* (2007) in which 200mg/kg bw of the extract had the highest glycemic change of 67% after 24hr extract administration compared with 400 and 800mg/kg bw of same extract that produced 60% glycemic change. Similarly, at all doses tested (25-100mg/kg bw) for *Artocarpus heterophyllus* leaf extract, the dose that exerted optimal effect was 50mg/kg bw. The same observation was true for trypanocidal activity of *Anona senegalensis* in which 200mg/kg bw dose had the best activity compared with 100,150 and 250 mg/kg bw extracts. It was also reported by Bamosa *etal* (2001) that out of all the doses (50,100,200,300,400 and 500 mg/kg bw) of *Nigella sativa* extracted and tested on rats, the most effective dose lies between 100 and 300 mg/kg bw. Rath *etal* (2002) evaluated the alcoholic extract of *Mucina pruriens* (100,200 and 400 mg/kg bw) in alloxan induced diabetic rats and streptozotocin induced diabetic mice and reported that the maximum effect occurred at a dose of 200 mg/kg bw.

The ethylacetate/ethanol fraction(Fig 4.34) was next interms of activity followed by ethylacetate fraction (Fig 4.33) at same dose while hexane fraction had the least activity. The significant glucose reduction may be due to bioactive compounds that mimicks the insulin action since the beta cells were destroyed

(Jegadeeson,2002). The polarity variation may also be responsible for the solubility of the active components in the different fractions. Generally, all the fractions of the *Anacardium* leaf extract at varying doses had no significant effect on the weight of the rats. This might be as a result of non stimulation of certain hormones or enzymes associated with weight (Tedong, 2006).

b. Fractions of Stem bark extract.

The ethylacetate fraction of the stem bark extract of the stem bark extract of *Anacardium* at 300mg/kg bw (Fig 4.45) had a better hypoglycemic activity compared with other fractions at the same dose. At 200mg/kg bw(Fig 4.37),the ethylacetate fraction had a better hypoglycemic activity than other fractions at same dose.Their activities may be linked to the polarity of the solvents. The activity observed for the fractions is similar to the activity of *Jatropha tanjorensis* leaf which acts by aiding glucose metabolism, its uptake and utilization in blood and body tissues rather than through β-cells (Olayiwola *etal*,2004).

5.1.6 Toxicological Studies of Ethanolic Extract of *A. occidentale* Leaf.

Toxicity studies are usually undertaken to define the and basic toxicity of a substance, access the susceptible species, identify target organs, provide data for risk assesement in case of acute exposure to the chemical or drug, provide information for the design and selection of dose levels for prolonged studies (Wallace,2001).

The daily food intake by the rats fed ethanolic extracts of *Anacardium* leaf significantly decreased for the period of study signifying that the extract might have

suppressed the metabolic activities of the rats. This is in agreement with findings by Tedong *etal*, 2007 in which there was decrease in food intake when rats were fed hexane extract of *A. occidentale* leaf.

The body weight revealed that the weight of the rats kept fluctuating which corroborates the food intake by the rats. This is in agreement with literature reports on rats administered hexane extract of *Anacardium occidentale* leaf (Theo *etal*, 2002). It was reported by Wilson *etal* (2001) that a weekly body weight measurement for mice is recommended under chronic toxicological assesement. Body weight is one of the most sensitive indicators of the condition of an animal. Together with food consumption, the two are the significant toxicological findings especially with materials of low toxicity. The data may indicate change in appetite or efficiency of food utilization by the body (EPA/OPPTS,1988). Stress, diarrhea, dehydration or decrease in water consumption are other factors that influences weight change. Therefore, certain phytoconstituents of Anacardium leaf extract may have effect on the appetite of the animals studied hence the fluctuation in their body weights (Rao and Knapoka,1987).

The packed cell volume (PCV) of the experimental rats (Fig 4.2) fluctuate which may be an indication that the fluid intake by the rats as a result of extract administration was irregular and there was no complete dehydration that will lead to constant increase in the PCV values. The same trend was observed for the red blood cells (Fig 4.3) and the white blood cells (Fig 4.4). These findings are in agreement with those reported by Tedong *etal* (2007). It was reported by Shermer(1967) that animals showed appreciable fluctuations in their haematological parameters as a result of changes in nutrition and/or the environment.

The liver enzymes (ALT and AST) significantly decreased (Table 4.9). The decreased levels of the enzymes suggests impaired protein synthesis process in the liver which is in agreement with the findings of other researchers (Chandan *etal*, 1991; Kamis *etal*,2003; Tedong *etal*,2007;www.essortment.com). Since there was decrease in the levels of liver enzymes at varying doses (Table 4.9), its reasonable to deduce that ethanolic extract of *Anacardium occidentale* may induce liver damage (Kamtchoung *etal*, 1998). Elevations in ALT levels are rarely observed except in chronic liver disease (Kachmar *etal*,1973; Gad,2001;Haschek and Rousseax,1991) while elevations in AST levels are usually associated with skeletal and heart muscles. The prognosis most atimes cannot be conclusive unless other parameters and histopathological evidences are considered (Loeb and Qainby,1989).

The significant increase observed for creatinine and urea may induce kidney damage in rats (Zaki and Dafallah, 2004). However, the significant decrease in the protein levels corroborates the decrease in enzyme levels. This is in agreement with the findings of Tedong *etal* (2006) in which rats were fed hexane extract of *Anacardium occidentale* leaf and a decrease in protein levels was observed.

5.1.7 Sub chronic toxicity of Ethanolic Extract of *A.occidentale* Leaf.

The extract significantly altered the structures of the liver, kidney and pancreas (Fig 4.54).This might be due to alterations in the activities of certain hormones responsible for growth (Gad,2001) . Although, the spleen remain unchanged, it was reported by Tedong *etal* (2007) that on administration of 14000

mg/kg bw of hexane extract of *A. occidentale* leaf to rats, the liver and kidney weights were slightly altered after 56 days which is in agreement with the findings in this study.

5.1.8 Comparison of the Hypoglycemic Activity of Purified Fractions of *A. occidentale* Leaf extract with Metformin.

The column fractions (Fig 4.55) were compared with the standard hypoglycemic drug (Metformin). Metformin is an oral antidiabetic drug of biguanide origin known to exert its effects in the liver thereby inhibiting gluconeogenesis. It is usually well tolerated with minimal side effects, though with some patients developing gastrointestinal side effects but exceedingly rare and effectively occurs only when it is used in severe renal failure (Nathan,2009; Haries *etal*,2009; Noor *etal*, 2009). The ethylacetate fraction gave the same hypoglycemic potential with the metformin drug which is an indication that the active principles in the fraction were soluble in ethylacetate in its purified state and may posses same antidiabetic effects but may differ in mode of action. Thus, the bioactive principles are soluble in intermediate polar solvent.

5.1.9 Spectral Studies of Purified Active Fraction (Ethylacetate) of *A. occidentale* Leaf extract.

The spectral studies (Gas chromatography- Mass spectroscopy and Infra red) revealed that the likely active hypoglycemic principle contained in the purified fraction are 1,2-benzenedicarboxylic acid,mono(2-ethylhexyl)ester and Bis (2-

ethylhexyl)phthalate. This is in agreement with the findings of Nageshwara and Mahesh(2000) who reported that the antidiabetic activity of *Casia auriculata* leaves was linked to di-(2-ethyl hexylphthalate). This also implies that the bioactive principles were isolated without loss of activity and hence crude extracts of A. occidentale leaf can be standardized and used as phytomedicine.

The two compounds detected are known to exert their effect by activating the gamma peroxisome proliferators activated receptors (gamma PPAR) of the rat liver which facilitates the binding of insulin to receptors hence sensitizes the glucose uptake into the cells. The two components are therefore insulin sensitizers. The same holds for the hypoglycemic drugs of thiolidinedione origin which are found in the market as pioglitazone (Actos) and rosiglitazone (Avantia). Although the administration of insulin sensitizers like thiolidinediones to diabetics are known to cause or induce toxicity and liver cancer through the activation of gamma PPARs, researches are being carried out to eliminate the problems or toxicity (Moses *etal*, 2004).

5.1.10 Histopathology of Organs

The hepatocellular alterations observed (PlatesII-IV) was probably due to the inhibition of the sites of enzyme synthesis in the liver leading to reduced enzyme activities. This finding however, corroborates the earlier decrease noted in total protein, ALT and AST levels. This is in agreement with findings of Tedong (2007) in which alterations were observed in liver tissues when rats were fed hexane extract of *A. occidentale* leaf.

The morphological changes observed in kidney tubules (Plates VI-VIII) is associated with increased urea and creatinine levels. Similar results were obtained with rabbits treated with *Eugenia jambolana* (Kedar and Chakrabarti, 1983) and *Bauhinia forticata* (Pepato *etal*,2002). The destruction of some glomeruli noted with the kidney tissues may likely be due to nephrotoxic effect of *A. occidentale* leaf extract on the tissues. A similar observation was reported by other workers in which the methanolic stem bark extract of *Steganotaenia araliacea* in rats showed relative hepatotoxicity at doses of 1000mg/kg bw pointing to its unsuitability for human therapy (Agunu *etal*, 2003).

5.1.11Antibacterial Activity of Fractions of *A. occidentale* Leaf extract.

The antibacterial activity of stepwise fractions of *Anacardium* (Table 4.6, Plates I-III) revealed that the antibacterial principles that inhibits *Salmonella typhi*(gram negative) are soluble in less polar solvent while that of *Staphylococcus aureus*(gram positive) are soluble in an intermediate polar solvent. However, the active principles that inhibits *Kliebsella pneumonae*(gram negative) resides in solvents of varying polarities. The activity of the fractions may be attributed to the presence and action of the phytochemicals. This is in agreement with reports of Ayepola and Adeniji (2008) on the antibacterial activity of leaf extracts of *Eucalyptus camaldulensis* in which it was reported that the activity of the extracts was due to the presence of the phytochemicals especially polyphenolic compounds and/or volatile oils.The mechanism of action of tannins is based on their ability to bind proteins

thereby inhibiting protein synthesis (Ayepola and Adeniyi,2008). It was reported by Kudi(1999) that *A. occidentale* had good invitro antibacterial activity against *E.coli* and *Pseudomonas* species. Akinpelu(2001) also reported that bark extract of *A. occidentale* exhibited invitro antimicrobial activity against 13 of 15 microbes tested. In the same study, extract of *A. occidentale* leaf was reported to exhibit activity against gram negative bacterium *Helicobacter pylori* which causes stomach ulcer.

5.2 SUMMARY AND CONCLUSIONS

1. In all, six plants were analyzed namely: *Moringa oleifera, Artemisia herba-alba, Zyzzipus spinachristi, Terminalia glaucescens, Anacardium occidentale* and *Blighia sapida.*
2. All the plants analyzed except *Zyzzipus spinachristi*, *Artemisia herba alba* and *Terminalia glaucesens* had more than 50% glucose reduction potentials.
3. All the plants are rich in phytochemicals.
4. *A. occidentale* posses the highest antidiabetic activity compared to other plants.
5. The bioactive components in *A. occidentale* contain esters which are known to be insulin sensitizers.
6. The mode of action of the active principles is by activation of gamma-PPARs which facilitates insulin recognition by receptors.
7. Histopathology of the kidney and liver tissues revealed lesions at *A.occidentale* doses ≥ 2000mg/kg bwt indicating its toxic effects.
8. The *A. occidentale* posses antibacterial activity when tested on some bacterial species namely : *Salmonella typhi* (gram-ve), *Staphyloccocus aureus* (gram +) and *Kliebsella pneumonae* (gram-ve).

5.3 SUGGESTIONS/RECOMMENDATIONS

1. More antidiabetic plants used as antidiabetic remedies should be screened.
2. Studies on animal trials should be extended to higher animals e.g rabbits,chimpazees, monkeys e.t.c.
3. The bioactive compounds obtained should be further investigated for possible drug development.

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APPENDICES

Appendix I-STATISTICAL ANALYSIS

T-Test (Week 0)

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	CONTROL Diabetic	3	91.4333	1.00664	.58119
	TREATED Diabetic	3	93.2667	.76376	.44096
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	CONTROL Diabetic	3	53.1000	3.46987	2.00333
	TREATED Diabetic	3	53.6333	2.28108	1.31698
Effect of M. oleifera extract on Serum triglyceride	CONTROL Diabetic	3	83.8000	2.91376	1.68226
	TREATED Diabetic	3	83.7333	2.51661	1.45297

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	Equal variances assumed	.193	.683	-2.513	4	.066	-1.83333	.72954	-3.85885	.19218
	Equal variances not assumed			-2.513	3.730	.070	-1.83333	.72954	-3.91812	.25146
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	Equal variances assumed	1.244	.327	-.222	4	.835	-.53333	2.39745	-7.18973	6.12306
	Equal variances not assumed			-.222	3.457	.836	-.53333	2.39745	-7.62342	6.55675
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	Equal variances assumed	.116	.750	.030	4	.978	.06667	2.22286	-6.10498	6.23832
	Equal variances not assumed			.030	3.917	.978	.06667	2.22286	-6.15685	6.29018

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	CONTROL Normoglycemic	3	74.0333	3.17857	1.83515
	TREATED Normoglycemic	3	78.0000	1.51327	.87369
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	CONTROL Normoglycemic	3	42.5667	1.81475	1.04775
	TREATED Normoglycemic	3	42.4333	2.65016	1.53007
Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats	CONTROL Normoglycemic	3	47.6667	1.48436	.85700
	TREATED Normoglycemic	3	48.3667	.90185	.52068
Effect of A. occidentale extract on weight (g) of normoglycemic rats	CONTROL Normoglycemic	3	125.6667	4.95412	2.86026
	TREATED Normoglycemic	3	153.4333	5.16946	2.98459
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	CONTROL Normoglycemic	3	84.1000	3.15753	1.82300
	TREATED Normoglycemic	3	84.7333	1.98578	1.14649

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	Equal variances assumed	2.992	.159	-1.952	4	.123	-3.96667	2.03251	-9.60983	1.67650
	Equal variances not assumed			-1.952	2.862	.150	-3.96667	2.03251	-10.61464	2.68131
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	Equal variances assumed	.727	.442	.072	4	.946	.13333	1.85442	-5.01537	5.28204
	Equal variances not assumed			.072	3.538	.947	.13333	1.85442	-5.29198	5.55865
Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats	Equal variances assumed	.994	.375	-.698	4	.524	-.70000	1.00277	-3.48415	2.08415
	Equal variances not assumed			-.698	3.299	.531	-.70000	1.00277	-3.73350	2.33350
Effect of A. occidentale extract on weight (g) of normoglycemic rats	Equal variances assumed	.104	.764	-6.717	4	.003	-27.76667	4.13387	-39.24413	-16.28920
	Equal variances not assumed			-6.717	3.993	.003	-27.76667	4.13387	-39.25232	-16.28101
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	Equal variances assumed	1.278	.321	-.294	4	.783	-.63333	2.15355	-6.61255	5.34588
	Equal variances not assumed			-.294	3.368	.786	-.63333	2.15355	-7.08207	5.81540

T-Test (Week 1)

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	CONTROL Normoglycemic	3	75.3667	.90185	.52068
	TREATED Normoglycemic	3	65.3333	4.17892	2.41270
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	CONTROL Normoglycemic	3	41.7333	.49329	.28480
	TREATED Normoglycemic	3	63.1667	3.02379	1.74579
Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats	CONTROL Normoglycemic	3	46.3667	1.05040	.60645
	TREATED Normoglycemic	3	57.4333	.95044	.54874
Effect of A. occidentale extract on weight (g) of normoglycemic rats	CONTROL Normoglycemic	3	127.1333	4.21940	2.43607
	TREATED Normoglycemic	3	153.4000	3.53695	2.04206
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	CONTROL Normoglycemic	3	86.1333	1.58219	.91348
	TREATED Normoglycemic	3	94.1333	3.10054	1.79010

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	Equal variances assumed	3.556	.132	4.065	4	.015	10.03333	2.46824	3.18039	16.88627
	Equal variances not assumed			4.065	2.186	.048	10.03333	2.46824	.23355	19.83312
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	Equal variances assumed	5.782	.074	-12.117	4	.000	-21.43333	1.76887	-26.34449	-16.52217
	Equal variances not assumed			-12.117	2.106	.006	-21.43333	1.76887	-28.68743	-14.17924
Effect of A. occidentale extract on Serum protein (g/l) in normoglycemic rats	Equal variances assumed	.021	.891	-13.531	4	.000	-11.06667	.81786	-13.33740	-8.79593
	Equal variances not assumed			-13.531	3.961	.000	-11.06667	.81786	-13.34632	-8.78701
Effect of A. occidentale extract on weight (g) of normoglycemic rats	Equal variances assumed	.177	.696	-8.263	4	.001	-26.26667	3.17875	-35.09229	-17.44104
	Equal variances not assumed			-8.263	3.882	.001	-26.26667	3.17875	-35.19944	-17.33389
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	Equal variances assumed	2.412	.195	-3.981	4	.016	-8.00000	2.00970	-13.57982	-2.42018
	Equal variances not assumed			-3.981	2.975	.029	-8.00000	2.00970	-14.42570	-1.57430

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	CONTROL Diabetic	3	94.3333	1.25033	.72188
	TREATED Diabetic	3	93.0333	3.08923	1.78357
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	CONTROL Diabetic	3	52.0333	1.40119	.80898
	TREATED Diabetic	3	64.5333	2.47857	1.43101
Effect of M. oleifera extract on Serum triglyceride	CONTROL Diabetic	3	85.1667	1.56312	.90247
	TREATED Diabetic	3	97.4667	1.05040	.60645

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	Equal variances assumed	4.532	.100	.676	4	.536	1.30000	1.92412	-4.04220	6.64220
	Equal variances not assumed			.676	2.638	.554	1.30000	1.92412	-5.32711	7.92711
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	Equal variances assumed	1.016	.371	-7.604	4	.002	-12.50000	1.64384	-17.06404	-7.93596
	Equal variances not assumed			-7.604	3.160	.004	-12.50000	1.64384	-17.58484	-7.41516
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	Equal variances assumed	.513	.514	-11.312	4	.000	-12.30000	1.08730	-15.31883	-9.28117
	Equal variances not assumed			-11.312	3.500	.001	-12.30000	1.08730	-15.49662	-9.10338

T-Test (week 2)

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	CONTROL Diabetic	3	95.1333	2.68390	1.54955
	TREATED Diabetic	3	71.6333	1.40119	.80898
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	CONTROL Diabetic	3	55.3333	1.05987	.61192
	TREATED Diabetic	3	64.0667	2.68390	1.54955
Effect of M. oleifera extract on Serum triglyceride	CONTROL Diabetic	3	83.3667	1.16762	.67412
	TREATED Diabetic	3	84.8667	2.19393	1.26667

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	Equal variances assumed	2.054	.225	13.444	4	.000	23.50000	1.74801	18.64673	28.35327
	Equal variances not assumed			13.444	3.015	.001	23.50000	1.74801	17.95250	29.04750
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	Equal variances assumed	3.602	.131	-5.242	4	.006	-8.73333	1.66600	-13.35889	-4.10778
	Equal variances not assumed			-5.242	2.609	.019	-8.73333	1.66600	-14.51445	-2.95221
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	Equal variances assumed	1.827	.248	-1.045	4	.355	-1.50000	1.43488	-5.48387	2.48387
	Equal variances not assumed			-1.045	3.049	.372	-1.50000	1.43488	-6.02535	3.02535

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	CONTROL Normoglycemic	3	75.4000	.90000	.51962
	TREATED Normoglycemic	3	63.8333	2.40069	1.38604
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	CONTROL Normoglycemic	3	43.5333	.85049	.49103
	TREATED Normoglycemic	3	63.6667	3.21299	1.85502
Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats	CONTROL Normoglycemic	3	47.4667	1.05040	.60645
	TREATED Normoglycemic	3	64.7333	1.49778	.86474
Effect of A. occidentale extract on weight (g) of normoglycemic rats	CONTROL Normoglycemic	3	138.7333	7.20162	4.15786
	TREATED Normoglycemic	3	149.9333	4.45234	2.57056
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	CONTROL Normoglycemic	3	85.1000	1.67033	.96437
	TREATED Normoglycemic	3	94.0333	2.63122	1.51914

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	Equal variances assumed	2.840	.167	7.814	4	.001	11.56667	1.48024	7.45686	15.67647
	Equal variances not assumed			7.814	2.551	.008	11.56667	1.48024	6.35065	16.78268
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	Equal variances assumed	5.842	.073	-10.492	4	.000	-20.13333	1.91891	-25.46109	-14.80558
	Equal variances not assumed			-10.492	2.279	.006	-20.13333	1.91891	-27.49317	-12.77350
Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats	Equal variances assumed	.652	.465	-16.348	4	.000	-17.26667	1.05620	-20.19914	-14.33419
	Equal variances not assumed			-16.348	3.584	.000	-17.26667	1.05620	-20.33834	-14.19499
Effect of A. occidentale extract on weight (g) of normoglycemic rats	Equal variances assumed	1.200	.335	-2.291	4	.084	-11.20000	4.88831	-24.77212	2.37212
	Equal variances not assumed			-2.291	3.334	.097	-11.20000	4.88831	-25.91122	3.51122
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	Equal variances assumed	.647	.466	-4.965	4	.008	-8.93333	1.79938	-13.92922	-3.93745
	Equal variances not assumed			-4.965	3.387	.012	-8.93333	1.79938	-14.30704	-3.55963

T-Test (week 3)

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	CONTROL Normoglycemic	3	75.3333	.90738	.52387
	TREATED Normoglycemic	3	61.2333	1.02632	.59255
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	CONTROL Normoglycemic	3	45.7000	1.94679	1.12398
	TREATED Normoglycemic	3	67.3667	.85049	.49103
Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats	CONTROL Normoglycemic	3	44.5333	1.58219	.91348
	TREATED Normoglycemic	3	67.1667	1.42945	.82529
Effect of A. occidentale extract on weight (g) of normoglycemic rats	CONTROL Normoglycemic	3	135.4667	8.29237	4.78760
	TREATED Normoglycemic	3	146.5333	4.27707	2.46937
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	CONTROL Normoglycemic	3	82.7333	2.51064	1.44952
	TREATED Normoglycemic	3	94.0333	1.72143	.99387

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	Equal variances assumed	.093	.775	17.827	4	.000	14.10000	.79092	11.90405	16.29595
	Equal variances not assumed			17.827	3.941	.000	14.10000	.79092	11.89098	16.30902
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	Equal variances assumed	3.016	.157	-17.665	4	.000	-21.66667	1.22656	-25.07214	-18.26120
	Equal variances not assumed			-17.665	2.737	.001	-21.66667	1.22656	-25.79154	-17.54179
Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats	Equal variances assumed	.041	.850	-18.385	4	.000	-22.63333	1.23108	-26.05136	-19.21531
	Equal variances not assumed			-18.385	3.959	.000	-22.63333	1.23108	-26.06520	-19.20147
Effect of A. occidentale extract on weight (g) of normoglycemic rats	Equal variances assumed	2.916	.163	-2.054	4	.109	-11.06667	5.38692	-26.02315	3.88982
	Equal variances not assumed			-2.054	2.994	.132	-11.06667	5.38692	-28.23035	6.09702
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	Equal variances assumed	.554	.498	-6.430	4	.003	-11.30000	1.75752	-16.17967	-6.42033
	Equal variances not assumed			-6.430	3.540	.005	-11.30000	1.75752	-16.44014	-6.15986

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	CONTROL Diabetic	3	93.2333	1.10604	.63857
	TREATED Diabetic	3	71.4000	.62450	.36056
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	CONTROL Diabetic	3	53.8333	2.43379	1.40515
	TREATED Diabetic	3	67.5333	2.01080	1.16094
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	CONTROL Diabetic	3	85.1000	1.57162	.90738
	TREATED Diabetic	3	78.0333	1.59478	.92075

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	Equal variances assumed	.766	.431	29.773	4	.000	21.83333	.73333	19.79727	23.86939
	Equal variances not assumed			29.773	3.158	.000	21.83333	.73333	19.56406	24.10261
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	Equal variances assumed	.106	.761	-7.516	4	.002	-13.70000	1.82270	-18.76062	-8.63938
	Equal variances not assumed			-7.516	3.863	.002	-13.70000	1.82270	-18.83243	-8.56757
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	Equal variances assumed	.006	.943	5.467	4	.005	7.06667	1.29271	3.47752	10.65582
	Equal variances not assumed			5.467	3.999	.005	7.06667	1.29271	3.47721	10.65612

T-Test (week 4)

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	CONTROL Diabetic	3	93.6333	1.33167	.76884
	TREATED Diabetic	3	66.1667	3.41516	1.97174
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	CONTROL Diabetic	3	55.5000	3.99625	2.30723
	TREATED Diabetic	3	66.7667	2.20303	1.27192
Effect of M. oleifera extract on Serum triglyceride	CONTROL Diabetic	3	83.9333	1.35769	.78387
	TREATED Diabetic	3	76.0333	1.35769	.78387

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of B. sapida ext on Serum Triglyceride level (mg/dl) in diabeti rats	Equal variance assumed	2.504	.189	12.978	4	.000	27.46667	2.11634	21.59077	33.34256
	Equal variance not assumed			12.978	2.594	.002	27.46667	2.11634	20.09490	34.83843
Effect of B. sapida ext on Serum Total protei (g/l) in diabetic rats	Equal variance assumed	.987	.377	-4.276	4	.013	-11.26667	2.63460	-18.58149	-3.95185
	Equal variance not assumed			-4.276	3.113	.022	-11.26667	2.63460	-19.48180	-3.05154
Effect of M. oleifera e on Serum triglyceride (mg/dl) of diabetic rat	Equal variance assumed	.000	1.000	7.126	4	.002	7.90000	1.10855	4.82216	10.97784
	Equal variance not assumed			7.126	4.000	.002	7.90000	1.10855	4.82216	10.97784

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	CONTROL Normoglycemic	3	74.2000	1.93132	1.11505
	TREATED Normoglycemic	3	61.3333	1.05987	.61192
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	CONTROL Normoglycemic	3	42.8000	.45826	.26458
	TREATED Normoglycemic	3	68.0333	.56862	.32830
Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats	CONTROL Normoglycemic	3	47.1333	1.35769	.78387
	TREATED Normoglycemic	3	67.4333	1.00167	.57831
Effect of A. occidentale extract on weight (g) of normoglycemic rats	CONTROL Normoglycemic	3	137.0333	5.58241	3.22301
	TREATED Normoglycemic	3	144.1333	5.47205	3.15929
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	CONTROL Normoglycemic	3	86.0000	1.41774	.81854
	TREATED Normoglycemic	3	95.1333	1.55349	.89691

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	Equal variances assumed	1.179	.339	10.116	4	.001	12.86667	1.27192	9.33525	16.39808
	Equal variances not assumed			10.116	3.104	.002	12.86667	1.27192	8.89483	16.83850
Effect of A. occidentale extract on Serum triglyceride level (mg/d in normoglycemic rats	Equal variances assumed	.239	.651	-59.846	4	.000	-25.23333	.42164	26.40399	24.06268
	Equal variances not assumed			-59.846	3.827	.000	-25.23333	.42164	26.42513	24.04154
Effect of A. occidentale extract on Serum prote (g/ll) in normoglycemic rats	Equal variances assumed	.761	.432	-20.840	4	.000	-20.30000	.97411	23.00456	17.59544
	Equal variances not assumed			-20.840	3.680	.000	-20.30000	.97411	23.10002	17.49998
Effect of A. occidentale extract on weight (g) o normoglycemic rats	Equal variances assumed	.005	.947	-1.573	4	.191	-7.10000	4.51319	19.63063	5.43063
	Equal variances not assumed			-1.573	3.998	.191	-7.10000	4.51319	19.63260	5.43260
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	Equal variances assumed	.033	.864	-7.522	4	.002	-9.13333	1.21427	12.50468	-5.76199
	Equal variances not assumed			-7.522	3.967	.002	-9.13333	1.21427	12.51576	-5.75090

Oneway (Control)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	.00	3	91.4333	1.00664	.58119	88.9327	93.9340	90.50	92.50
	1.00	3	94.3333	1.25033	.72188	91.2273	97.4393	93.10	95.60
	2.00	3	95.1333	2.68390	1.54955	88.4661	101.8005	92.10	97.20
	3.00	3	93.2333	1.10604	.63857	90.4858	95.9809	92.20	94.40
	4.00	3	93.6333	1.33167	.76884	90.3253	96.9414	92.10	94.50
	Total	15	93.5533	1.86466	.48145	92.5207	94.5859	90.50	97.20
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	.00	3	53.1000	3.46987	2.00333	44.4804	61.7196	50.90	57.10
	1.00	3	52.0333	1.40119	.80898	48.5526	55.5141	50.60	53.40
	2.00	3	55.3333	1.05987	.61192	52.7005	57.9662	54.20	56.30
	3.00	3	53.8333	2.43379	1.40515	47.7875	59.8792	51.20	56.00
	4.00	3	55.5000	3.99625	2.30723	45.5728	65.4272	51.20	59.10
	Total	15	53.9600	2.67550	.69081	52.4784	55.4416	50.60	59.10
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	.00	3	74.0333	3.17857	1.83515	66.1373	81.9293	70.40	76.30
	1.00	3	75.3667	.90185	.52068	73.1263	77.6070	74.50	76.30
	2.00	3	75.4000	.90000	.51962	73.1643	77.6357	74.50	76.30
	3.00	3	75.3333	.90738	.52387	73.0793	77.5874	74.50	76.30
	4.00	3	74.2000	1.93132	1.11505	69.4023	78.9977	72.10	75.90
	Total	15	74.8667	1.65256	.42669	73.9515	75.7818	70.40	76.30
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	.00	3	42.5667	1.81475	1.04775	38.0586	47.0748	40.90	44.50
	1.00	3	41.7333	.49329	.28480	40.5079	42.9587	41.40	42.30
	2.00	3	43.5333	.85049	.49103	41.4206	45.6461	42.90	44.50
	3.00	3	45.7000	1.94679	1.12398	40.8639	50.5361	43.50	47.20
	4.00	3	42.8000	.45826	.26458	41.6616	43.9384	42.30	43.20
	Total	15	43.2667	1.76622	.45604	42.2886	44.2448	40.90	47.20
Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats	.00	3	47.6667	1.48436	.85700	43.9793	51.3540	46.40	49.30
	1.00	3	46.3667	1.05040	.60645	43.7573	48.9760	45.30	47.40
	2.00	3	47.4667	1.05040	.60645	44.8573	50.0760	46.40	48.50
	3.00	3	44.5333	1.58219	.91348	40.6029	48.4637	42.80	45.90
	4.00	3	47.1333	1.35769	.78387	43.7606	50.5060	46.30	48.70
	Total	15	46.6333	1.62554	.41971	45 7331	47.5335	42.80	49.30
Effect of A. occidentale extract on weight (g) of normoglycemic rats	.00	3	125.6667	4.95412	2.86026	113.3599	137.9734	120.60	130.50
	1.00	3	127.1333	4.21940	2.43607	116.6518	137.6149	122.40	130.50
	2.00	3	138.7333	7.20162	4.15786	120.8435	156.6231	130.60	144.30
	3.00	3	135.4667	8.29237	4.78760	114.8673	156.0660	125.90	140.60
	4.00	3	137.0333	5.58241	3.22301	123.1659	150.9008	130.60	140.60
	Total	15	132.8067	7.64372	1.97360	128.5737	137.0396	120.60	144.30
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	.00	3	84.1000	3.15753	1.82300	76.2563	91.9437	80.50	86.40
	1.00	3	86.1333	1.58219	.91348	82.2029	90.0637	84.40	87.50
	2.00	3	85.1000	1.67033	.96437	80.9507	89.2493	83.30	86.60
	3.00	3	82.7333	2.51064	1.44952	76.4965	88.9701	80.60	85.50
	4.00	3	86.0000	1.41774	.81854	82.4781	89.5219	84.40	87.10
	Total	15	84.8133	2.25764	.58292	83.5631	86.0636	80.50	87.50
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	.00	3	83.8000	2.91376	1.68226	76.5618	91.0382	80.60	86.30
	1.00	3	85.1667	1.56312	.90247	81.2837	89.0497	83.50	86.60
	2.00	3	83.3667	1.16762	.67412	80.4661	86.2672	82.10	84.40
	3.00	3	85.1000	1.57162	.90738	81.1959	89.0041	83.40	86.50
	4.00	3	83.9333	1.35769	.78387	80.5606	87.3060	82.50	85.20
	Total	15	84.2733	1.71442	.44266	83.3239	85.2227	80.60	86.60

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	Between Groups	23.124	4	5.781	2.262	.135
	Within Groups	25.553	10	2.555		
	Total	48.677	14			
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	Between Groups	26.176	4	6.544	.884	.508
	Within Groups	74.040	10	7.404		
	Total	100.216	14			
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	Between Groups	5.673	4	1.418	.436	.780
	Within Groups	32.560	10	3.256		
	Total	38.233	14			
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	Between Groups	27.153	4	6.788	4.109	.032
	Within Groups	16.520	10	1.652		
	Total	43.673	14			
Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats	Between Groups	19.480	4	4.870	2.781	.086
	Within Groups	17.513	10	1.751		
	Total	36.993	14			
Effect of A. occidentale extract on weight (g) of normoglycemic rats	Between Groups	429.696	4	107.424	2.767	.087
	Within Groups	388.273	10	38.827		
	Total	817.969	14			
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	Between Groups	24.204	4	6.051	1.283	.340
	Within Groups	47.153	10	4.715		
	Total	71.357	14			
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	Between Groups	7.929	4	1.982	.597	.673
	Within Groups	33.220	10	3.322		
	Total	41.149	14			

Post Hoc Tests

Homogeneous Subsets

Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats

Duncan[a]

Week	N	Subset for alpha = .05	
		1	2
.00	3	91.4333	
3.00	3	93.2333	93.2333
4.00	3	93.6333	93.6333
1.00	3	94.3333	94.3333
2.00	3		95.1333
Sig.		.065	.204

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats

Duncan[a]

Week	N	Subset for alpha = .05	
		1	2
1.00	3	41.7333	
.00	3	42.5667	
4.00	3	42.8000	
2.00	3	43.5333	43.5333
3.00	3		45.7000
Sig.		.141	.066

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats

Duncan[a]

Week	N	Subset for alpha = .05	
		1	2
3.00	3	44.5333	
1.00	3	46.3667	46.3667
4.00	3		47.1333
2.00	3		47.4667
.00	3		47.6667
Sig.		.121	.288

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Effect of A. occidentale extract on weight (g) of normoglycemic rats

Duncan[a]

Week	N	Subset for alpha = .05	
		1	2
.00	3	125.6667	
1.00	3	127.1333	127.1333
3.00	3	135.4667	135.4667
4.00	3	137.0333	137.0333
2.00	3		138.7333
Sig.		.064	.059

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Oneway (Treated)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	.00	3	93.2667	.76376	.44096	91.3694	95.1640	92.60	94.10
	1.00	3	93.0333	3.08923	1.78357	85.3593	100.7074	91.20	96.60
	2.00	3	71.6333	1.40119	.80898	68.1526	75.1141	70.50	73.20
	3.00	3	71.4000	.62450	.36056	69.8487	72.9513	70.90	72.10
	4.00	3	66.1667	3.41516	1.97174	57.6829	74.6504	63.20	69.90
	Total	15	79.1000	12.18817	3.14697	72.3504	85.8496	63.20	96.60
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	.00	3	53.6333	2.28108	1.31698	47.9668	59.2999	51.60	56.10
	1.00	3	64.5333	2.47857	1.43101	58.3762	70.6905	62.30	67.20
	2.00	3	64.0667	2.68390	1.54955	57.3995	70.7339	62.00	67.10
	3.00	3	67.5333	2.01080	1.16094	62.5382	72.5284	65.30	69.20
	4.00	3	66.7667	2.20303	1.27192	61.2940	72.2393	65.30	69.30
	Total	15	63.3067	5.55086	1.43323	60.2327	66.3806	51.60	69.30
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	.00	3	78.0000	1.51327	.87369	74.2408	81.7592	76.30	79.20
	1.00	3	65.3333	4.17892	2.41270	54.9523	75.7143	60.90	69.20
	2.00	3	63.8333	2.40069	1.38604	57.8697	69.7970	61.20	65.90
	3.00	3	61.2333	1.02632	.59255	58.6838	63.7829	60.10	62.10
	4.00	3	61.3333	1.05987	.61192	58.7005	63.9662	60.20	62.30
	Total	15	65.9467	6.74144	1.74063	62.2134	69.6799	60.10	79.20
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	.00	3	42.4333	2.65016	1.53007	35.8500	49.0167	40.30	45.40
	1.00	3	63.1667	3.02379	1.74579	55.6551	70.6782	60.60	66.50
	2.00	3	63.6667	3.21299	1.85502	55.6851	71.6482	61.20	67.30
	3.00	3	67.3667	.85049	.49103	65.2539	69.4794	66.50	68.20
	4.00	3	68.0333	.56862	.32830	66.6208	69.4459	67.40	68.50
	Total	15	60.9333	9.98067	2.57700	55.4062	66.4604	40.30	68.50
Effect of A. occidentale extract on Serum protein (g/l) in normoglycemic rats	.00	3	48.3667	.90185	.52068	46.1263	50.6070	47.50	49.30
	1.00	3	57.4333	.95044	.54874	55.0723	59.7944	56.50	58.40
	2.00	3	64.7333	1.49778	.86474	61.0127	68.4540	63.50	66.40
	3.00	3	67.1667	1.42945	.82529	63.6157	70.7176	65.60	68.40
	4.00	3	67.4333	1.00167	.57831	64.9451	69.9216	66.40	68.40
	Total	15	61.0267	7.61122	1.96521	56.8117	65.2416	47.50	68.40
Effect of A. occidentale extract on weight (g) of normoglycemic rats	.00	3	153.4333	5.16946	2.98459	140.5917	166.2750	150.30	159.40
	1.00	3	153.4000	3.53695	2.04206	144.6137	162.1863	149.50	156.40
	2.00	3	149.9333	4.45234	2.57056	138.8731	160.9936	146.30	154.90
	3.00	3	146.5333	4.27707	2.46937	135.9085	157.1582	141.60	149.20
	4.00	3	144.1333	5.47205	3.15929	130.5400	157.7267	140.30	150.40
	Total	15	149.4867	5.47721	1.41421	146.4535	152.5198	140.30	159.40
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	.00	3	84.7333	1.98578	1.14649	79.8004	89.6663	82.50	86.30
	1.00	3	94.1333	3.10054	1.79010	86.4312	101.8355	90.60	96.40
	2.00	3	94.0333	2.63122	1.51914	87.4970	100.5697	91.20	96.40
	3.00	3	94.0333	1.72143	.99387	89.7571	98.3096	92.10	95.40
	4.00	3	95.1333	1.55349	.89691	91.2742	98.9924	93.40	96.40
	Total	15	92.4133	4.43587	1.14534	89.9568	94.8698	82.50	96.40
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	.00	3	83.7333	2.51661	1.45297	77.4817	89.9849	81.40	86.40
	1.00	3	97.4667	1.05040	.60645	94.8573	100.0760	96.40	98.50
	2.00	3	84.8667	2.19393	1.26667	79.4166	90.3167	82.40	86.60
	3.00	3	78.0333	1.59478	.92075	74.0717	81.9950	76.70	79.80
	4.00	3	76.0333	1.35769	.78387	72.6606	79.4060	74.60	77.30
	Total	15	84.0267	7.91468	2.04356	79.6437	88.4097	74.60	98.50

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	Between Groups	2031.433	4	507.858	105.176	.000
	Within Groups	48.287	10	4.829		
	Total	2079.720	14			
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	Between Groups	376.476	4	94.119	17.146	.000
	Within Groups	54.893	10	5.489		
	Total	431.369	14			
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	Between Groups	580.871	4	145.218	26.219	.000
	Within Groups	55.387	10	5.539		
	Total	636.257	14			
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	Between Groups	1339.520	4	334.880	60.806	.000
	Within Groups	55.073	10	5.507		
	Total	1394.593	14			
Effect of A. occidentale extract on Serum protein (g/l) in normoglycemic rats	Between Groups	797.016	4	199.254	142.189	.000
	Within Groups	14.013	10	1.401		
	Total	811.029	14			
Effect of A. occidentale extract on weight (g) of normoglycemic rats	Between Groups	205.411	4	51.353	2.393	.120
	Within Groups	214.587	10	21.459		
	Total	419.997	14			
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	Between Groups	223.764	4	55.941	10.818	.001
	Within Groups	51.713	10	5.171		
	Total	275.477	14			
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	Between Groups	843.716	4	210.929	63.393	.000
	Within Groups	33.273	10	3.327		
	Total	876.989	14			

Post Hoc Tests

Homogeneous Subsets

Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats

Duncan[a]

Week	N	Subset for alpha = .05		
		1	2	3
4.00	3	66.1667		
3.00	3		71.4000	
2.00	3		71.6333	
1.00	3			93.0333
.00	3			93.2667
Sig.		1.000	.899	.899

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats

Duncan[a]

Week	N	Subset for alpha = .05	
		1	2
.00	3	53.6333	
2.00	3		64.0667
1.00	3		64.5333
4.00	3		66.7667
3.00	3		67.5333
Sig.		1.000	.122

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats

Duncan[a]

Week	N	Subset for alpha = .05	
		1	2
3.00	3	61.2333	
4.00	3	61.3333	
2.00	3	63.8333	
1.00	3	65.3333	
.00	3		78.0000
Sig.		.075	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats

Duncan[a]

Week	N	Subset for alpha = .05		
		1	2	3
.00	3	42.4333		
1.00	3		63.1667	
2.00	3		63.6667	63.6667
3.00	3		67.3667	67.3667
4.00	3			68.0333
Sig.		1.000	.062	.054

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats

Duncan[a]

Week	N	Subset for alpha = .05			
		1	2	3	4
.00	3	48.3667			
1.00	3		57.4333		
2.00	3			64.7333	
3.00	3				67.1667
4.00	3				67.4333
Sig.		1.000	1.000	1.000	.788

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Effect of A. occidentale extract on weight (g) of normoglycemic rats

Duncan[a]

Week	N	Subset for alpha = .05	
		1	2
4.00	3	144.1333	
3.00	3	146.5333	146.5333
2.00	3	149.9333	149.9333
1.00	3		153.4000
.00	3		153.4333
Sig.		.174	.120

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats

Duncan[a]

Week	N	Subset for alpha = .05	
		1	2
.00	3	84.7333	
2.00	3		94.0333
3.00	3		94.0333
1.00	3		94.1333
4.00	3		95.1333
Sig.		1.000	.592

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats

Duncan[a]

Week	N	Subset for alpha = .05		
		1	2	3
4.00	3	76.0333		
3.00	3	78.0333		
.00	3		83.7333	
2.00	3		84.8667	
1.00	3			97.4667
Sig.		.209	.464	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

General Linear Model

Warnings

Post hoc tests are not performed for Sample because there are fewer than three groups.

Between-Subjects Factors

		Value Label	N
Sample	1.00	CONTROL Diabetic	15
	2.00	TREATED Diabetic	15
Week	.00		6
	1.00		6
	2.00		6
	3.00		6
	4.00		6

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	3621.299[a]	9	402.367	108.983	.000
	Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	1057.853[b]	9	117.539	18.233	.000
	Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	852.102[c]	9	94.678	28.477	.000
Intercept	Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	223568.801	1	223568.801	60554.930	.000
	Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	103136.033	1	103136.033	15998.351	.000
	Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	212436.675	1	212436.675	63897.135	.000
Sample	Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	1566.741	1	1566.741	424.361	.000
	Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	655.201	1	655.201	101.634	.000
	Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	.456	1	.456	.137	.715
Week	Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	938.679	4	234.670	63.562	.000
	Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	236.703	4	59.176	9.179	.000
	Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	453.263	4	113.316	34.083	.000
Sample * Week	Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	1115.879	4	278.970	75.561	.000
	Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	165.949	4	41.487	6.435	.002
	Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	398.382	4	99.595	29.957	.000
Error	Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	73.840	20	3.692		
	Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	128.933	20	6.447		
	Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	66.493	20	3.325		
Total	Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	227263.940	30			
	Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	104322.820	30			
	Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	213355.270	30			
Corrected Total	Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	3695.139	29			
	Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	1186.787	29			
	Effect of M. oleifera extract on Serum triglyceride	918.595	29			

Estimated Marginal Means

Grand Mean

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats	86.327	.351	85.595	87.058
Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats	58.633	.464	57.666	59.600
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats	84.150	.333	83.456	84.844

Post Hoc Tests

Week

Homogeneous Subsets

Effect of B. sapida extract on Serum Triglyceride level (mg/dl) in diabetic rats

Duncan[a,b,c]

Week	N	Subset		
		1	2	3
4.00	6	79.9000		
3.00	6		82.3167	
2.00	6		83.3833	
.00	6			92.3500
1.00	6			93.6833
Sig.		1.000	.348	.243

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 3.692.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Effect of B. sapida extract on Serum Total protein (g/l) in diabetic rats

Duncan[a,b,c]

Week	N	Subset 1	Subset 2
.00	6	53.3667	
1.00	6		58.2833
2.00	6		59.7000
3.00	6		60.6833
4.00	6		61.1333
Sig.		1.000	.088

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 6.447.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Effect of M. oleifera extract on Serum triglyceride (mg/dl) of diabetic rats

Duncan[a,b,c]

Week	N	Subset 1	Subset 2	Subset 3
4.00	6	79.9833		
3.00	6	81.5667		
.00	6		83.7667	
2.00	6		84.1167	
1.00	6			91.3167
Sig.		.148	.743	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 3.325.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

General Linear Model

Warnings

Post hoc tests are not performed for Sample because there are fewer than three groups.

Between-Subjects Factors

		Value Label	N
Sample	1.00	CONTROL Normoglycemic	15
	2.00	TREATED Normoglycemic	15
Week	.00		6
	1.00		6
	2.00		6
	3.00		6
	4.00		6

Descriptive Statistics

	Sample	Week	Mean	Std. Deviation	N
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	CONTROL Normoglycemic	.00	74.0333	3.17857	3
		1.00	75.3667	.90185	3
		2.00	75.4000	.90000	3
		3.00	75.3333	.90738	3
		4.00	74.2000	1.93132	3
		Total	74.8667	1.65256	15
	TREATED Normoglycemic	.00	78.0000	1.51327	3
		1.00	65.3333	4.17892	3
		2.00	63.8333	2.40069	3
		3.00	61.2333	1.02632	3
		4.00	61.3333	1.05987	3
		Total	65.9467	6.74144	15
	Total	.00	76.0167	3.11089	6
		1.00	70.3500	6.12462	6
		2.00	69.6167	6.53955	6
		3.00	68.2833	7.77134	6
		4.00	67.7667	7.18378	6
		Total	70.4067	6.62086	30
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	CONTROL Normoglycemic	.00	42.5667	1.81475	3
		1.00	41.7333	.49329	3
		2.00	43.5333	.85049	3
		3.00	45.7000	1.94679	3
		4.00	42.8000	.45826	3
		Total	43.2667	1.76622	15
	TREATED Normoglycemic	.00	42.4333	2.65016	3
		1.00	63.1667	3.02379	3
		2.00	63.6667	3.21299	3
		3.00	67.3667	.85049	3
		4.00	68.0333	.56862	3
		Total	60.9333	9.98067	15
	Total	.00	42.5000	2.03273	6
		1.00	52.4500	11.89836	6
		2.00	53.6000	11.22604	6
		3.00	56.5333	11.94314	6
		4.00	55.4167	13.82858	6
		Total	52.1000	11.41551	30
Effect of A. occidentale extract on Serum protein (g/l) in normoglycemic rats	CONTROL Normoglycemic	.00	47.6667	1.48436	3
		1.00	46.3667	1.05040	3
		2.00	47.4667	1.05040	3
		3.00	44.5333	1.58219	3
		4.00	47.1333	1.35769	3
		Total	46.6333	1.62554	15
	TREATED Normoglycemic	.00	48.3667	.90185	3
		1.00	57.4333	.95044	3
		2.00	64.7333	1.49778	3
		3.00	67.1667	1.42945	3
		4.00	67.4333	1.00167	3
		Total	61.0267	7.61122	15
	Total	.00	48.0167	1.16347	6
		1.00	51.9000	6.12732	6
		2.00	56.1000	9.52785	6
		3.00	55.8500	12.46992	6
		4.00	57.2833	11.16986	6
		Total	53.8300	9.10056	30
Effect of A. occidentale extract on weight (g) of normoglycemic rats	CONTROL Normoglycemic	.00	125.6667	4.95412	3
		1.00	127.1333	4.21940	3
		2.00	138.7333	7.20162	3
		3.00	135.4667	8.29237	3
		4.00	137.0333	5.58241	3
		Total	132.8067	7.64372	15
	TREATED Normoglycemic	.00	153.4333	5.16946	3
		1.00	153.4000	3.53695	3
		2.00	149.9333	4.45234	3
		3.00	146.5333	4.27707	3
		4.00	144.1333	5.47205	3
		Total	149.4867	5.47721	15
	Total	.00	139.5500	15.86836	6
		1.00	140.2667	14.80225	6
		2.00	144.3333	8.14289	6
		3.00	141.0000	8.45955	6
		4.00	140.5833	6.29012	6
		Total	141.1467	10.70713	30
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	CONTROL Normoglycemic	.00	84.1000	3.15753	3
		1.00	86.1333	1.58219	3
		2.00	85.1000	1.67033	3
		3.00	82.7333	2.51064	3
		4.00	86.0000	1.41774	3
		Total	84.8133	2.25764	15
	TREATED Normoglycemic	.00	84.7333	1.98578	3
		1.00	94.1333	3.10054	3
		2.00	94.0333	2.63122	3
		3.00	94.0333	1.72143	3
		4.00	95.1333	1.55349	3
		Total	92.4133	4.43587	15
	Total	.00	84.4167	2.38446	6
		1.00	90.1333	4.90374	6
		2.00	89.5667	5.27510	6
		3.00	88.3833	6.48180	6
		4.00	90.5667	5.17636	6
		Total	88.6133	5.18630	30

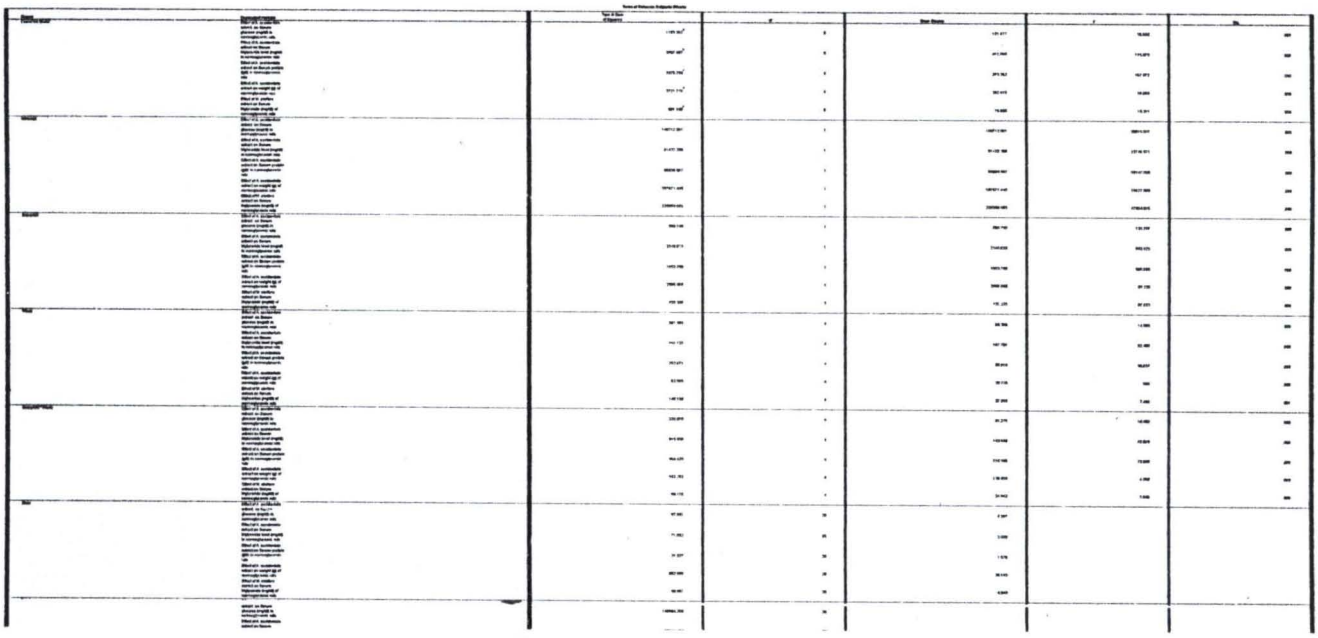
Multivariate Tests[c]

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	1.000	27784.672[a]	5.000	16.000	.000
	Wilks' Lambda	.000	27784.672[a]	5.000	16.000	.000
	Hotelling's Trace	8682.710	27784.672[a]	5.000	16.000	.000
	Roy's Largest Root	8682.710	27784.672[a]	5.000	16.000	.000
SampleN	Pillai's Trace	.992	373.686[a]	5.000	16.000	.000
	Wilks' Lambda	.008	373.686[a]	5.000	16.000	.000
	Hotelling's Trace	116.777	373.686[a]	5.000	16.000	.000
	Roy's Largest Root	116.777	373.686[a]	5.000	16.000	.000
Week	Pillai's Trace	2.212	4.701	20.000	76.000	.000
	Wilks' Lambda	.005	10.664	20.000	54.016	.000
	Hotelling's Trace	33.192	24.064	20.000	58.000	.000
	Roy's Largest Root	30.157	114.595[b]	5.000	19.000	.000
SampleN * Week	Pillai's Trace	2.017	3.863	20.000	76.000	.000
	Wilks' Lambda	.005	10.363	20.000	54.016	.000
	Hotelling's Trace	37.651	27.297	20.000	58.000	.000
	Roy's Largest Root	34.614	131.532[b]	5.000	19.000	.000

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

c. Design: Intercept+SampleN+Week+SampleN * Week



Estimated Marginal Means

Grand Mean

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats	70.407	.383	69.608	71.205
Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats	52.100	.345	51.379	52.821
Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats	53.830	.229	53.352	54.308
Effect of A. occidentale extract on weight (g) of normoglycemic rats	141.147	1.002	139.056	143.238
Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats	88.613	.406	87.767	89.460

Post Hoc Tests

Week

Homogeneous Subsets

Effect of A. occidentale extract on Serum glucose (mg/dl) in normoglycemic rats

Duncan[a,b,c]

Week	N	Subset 1	Subset 2
4.00	6	67.7667	
3.00	6	68.2833	
2.00	6	69.6167	
1.00	6	70.3500	
.00	6		76.0167
Sig.		.063	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 4.397.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Effect of A. occidentale extract on Serum triglyceride level (mg/dl) in normoglycemic rats

Duncan[a,b,c]

Week	N	Subset 1	Subset 2	Subset 3	Subset 4
.00	6	42.5000			
1.00	6		52.4500		
2.00	6		53.6000	53.6000	
4.00	6			55.4167	55.4167
3.00	6				56.5333
Sig.		1.000	.305	.112	.319

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 3.580.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Effect of A. occidentale extract on Serum protein (g/ll) in normoglycemic rats

Duncan[a,b,c]

Week	N	Subset 1	Subset 2	Subset 3
.00	6	48.0167		
1.00	6		51.9000	
3.00	6			55.8500
2.00	6			56.1000
4.00	6			57.2833
Sig.		1.000	1.000	.075

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 1.576.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Effect of A. occidentale extract on weight (g) of normoglycemic rats

Duncan[a,b,c]

Week	N	Subset 1
.00	6	139.5500
1.00	6	140.2667
4.00	6	140.5833
3.00	6	141.0000
2.00	6	144.3333
Sig.		.190

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 30.143.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Effect of M. oleifera extract on Serum triglyceride (mg/dl) of normoglycemic rats

Duncan[a,b,c]

Week	N	Subset 1	Subset 2
.00	6	84.4167	
3.00	6		88.3833
2.00	6		89.5667
1.00	6		90.1333
4.00	6		90.5667
Sig.		1.000	.133

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 4.943.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Oneway (Day 0)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Body weight (g)	Control	2	177.2400	3.57796	2.53000	145.0933	209.3867	174.71	179.77
	Group I (2000mg)	2	179.1400	2.14960	1.52000	159.8266	198.4534	177.62	180.66
	Group II (4000mg)	2	198.9400	2.37588	1.68000	177.5936	220.2864	197.26	200.62
	Group III (6000mg)	2	194.1600	8.71156	6.16000	115.8898	272.4302	188.00	200.32
	Total	8	187.3700	10.68775	3.77869	178.4348	196.3052	174.71	200.62
PVC (%)	Control	2	41.5000	.70711	.50000	35.1469	47.8531	41.00	42.00
	Group I (2000mg)	2	43.5000	.70711	.50000	37.1469	49.8531	43.00	44.00
	Group II (4000mg)	2	42.5000	.70711	.50000	36.1469	48.8531	42.00	43.00
	Group III (6000mg)	2	41.5000	.70711	.50000	35.1469	47.8531	41.00	42.00
	Total	8	42.2500	1.03510	.36596	41.3846	43.1154	41.00	44.00
RBC (x10**12 cells/L)	Control	2	7.6500	.49497	.35000	3.2028	12.0972	7.30	8.00
	Group I (2000mg)	2	7.8050	.48790	.34500	3.4214	12.1886	7.46	8.15
	Group II (4000mg)	2	8.0900	.19799	.14000	6.3111	9.8689	7.95	8.23
	Group III (6000mg)	2	8.8200	.26870	.19000	6.4058	11.2342	8.63	9.01
	Total	8	8.0913	.56187	.19865	7.6215	8.5610	7.30	9.01
WBC (x10**12 cells/L)	Control	2	7.4000	.28284	.20000	4.8588	9.9412	7.20	7.60
	Group I (2000mg)	2	7.0500	.77782	.55000	.0616	14.0384	6.50	7.60
	Group II (4000mg)	2	7.3500	.49497	.35000	2.9028	11.7972	7.00	7.70
	Group III (6000mg)	2	7.9000	.56569	.40000	2.8175	12.9825	7.50	8.30
	Total	8	7.4250	.53385	.18875	6.9787	7.8713	6.50	8.30

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Body weight (g)	Between Groups	700.638	3	233.546	9.440	.028
	Within Groups	98.959	4	24.740		
	Total	799.596	7			
PVC (%)	Between Groups	5.500	3	1.833	3.667	.121
	Within Groups	2.000	4	.500		
	Total	7.500	7			
RBC (x10**12 cells/L)	Between Groups	1.615	3	.538	3.623	.123
	Within Groups	.594	4	.149		
	Total	2.210	7			
WBC (x10**12 cells/L)	Between Groups	.745	3	.248	.795	.557
	Within Groups	1.250	4	.313		
	Total	1.995	7			

Post Hoc Tests

Homogeneous Subsets

Body weight (g)

Duncan[a]

Sample	N	Subset for alpha = .05	
		1	2
Control	2	177.2400	
Group I (2000mg)	2	179.1400	
Group III (6000mg)	2		194.1600
Group II (4000mg)	2		198.9400
Sig.		.722	.391

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

RBC (x1012 cells/L)**

Duncan[a]

Sample	N	Subset for alpha = .05	
		1	2
Control	2	7.6500	
Group I (2000mg)	2	7.8050	7.8050
Group II (4000mg)	2	8.0900	8.0900
Group III (6000mg)	2		8.8200
Sig.		.323	.061

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Oneway (Day 7)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Body weight (g)	Control	2	178.3000	.53740	.38000	173.4716	183.1284	177.92	178.68
	Group I (2000mg)	2	179.2950	7.03571	4.97500	116.0816	242.5084	174.32	184.27
	Group II (4000mg	2	200.7700	2.48902	1.76000	178.4071	223.1329	199.01	202.53
	Group III (6000mg	2	190.6600	6.15183	4.35000	135.3880	245.9320	186.31	195.01
	Total	8	187.2563	10.48425	3.70674	178.4912	196.0213	174.32	202.53
PVC (%)	Control	2	41.0000	1.41421	1.00000	28.2938	53.7062	40.00	42.00
	Group I (2000mg)	2	43.0000	.00000	.00000	43.0000	43.0000	43.00	43.00
	Group II (4000mg	2	44.0000	.00000	.00000	44.0000	44.0000	44.00	44.00
	Group III (6000mg	2	41.5000	2.12132	1.50000	22.4407	60.5593	40.00	43.00
	Total	8	42.3750	1.59799	.56497	41.0390	43.7110	40.00	44.00
RBC (x10**12 cells/L	Control	2	7.6150	1.25158	.88500	-3.6300	18.8600	6.73	8.50
	Group I (2000mg)	2	7.7750	1.61927	1.14500	-6.7736	22.3236	6.63	8.92
	Group II (4000mg	2	7.4250	.74246	.52500	.7542	14.0958	6.90	7.95
	Group III (6000mg	2	8.5150	.12021	.08500	7.4350	9.5950	8.43	8.60
	Total	8	7.8325	.93497	.33056	7.0508	8.6142	6.63	8.92
WBC (x10**12 cells/	Control	2	7.4500	.63640	.45000	1.7322	13.1678	7.00	7.90
	Group I (2000mg)	2	7.5000	.70711	.50000	1.1469	13.8531	7.00	8.00
	Group II (4000mg	2	7.7500	.49497	.35000	3.3028	12.1972	7.40	8.10
	Group III (6000mg	2	8.2000	.56569	.40000	3.1175	13.2825	7.80	8.60
	Total	8	7.7250	.55742	.19708	7.2590	8.1910	7.00	8.60

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Body weight (g)	Between Groups	675.606	3	225.202	9.600	.027
	Within Groups	93.830	4	23.458		
	Total	769.436	7			
PVC (%)	Between Groups	11.375	3	3.792	2.333	.215
	Within Groups	6.500	4	1.625		
	Total	17.875	7			
RBC (x10**12 cells/L)	Between Groups	1.365	3	.455	.383	.772
	Within Groups	4.754	4	1.189		
	Total	6.119	7			
WBC (x10**12 cells/L)	Between Groups	.705	3	.235	.639	.628
	Within Groups	1.470	4	.368		
	Total	2.175	7			

Post Hoc Tests

Homogeneous Subsets

Body weight (g)

Duncan[a]

Sample	N	Subset for alpha = .05	
		1	2
Control	2	178.3000	
Group I (2000mg)	2	179.2950	
Group III (6000mg)	2	190.6600	190.6600
Group II (4000mg)	2		200.7700
Sig.		.067	.105

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

PVC (%)

Duncan[a]

Sample	N	Subset for alpha = .05
		1
Control	2	41.0000
Group III (6000mg)	2	41.5000
Group I (2000mg)	2	43.0000
Group II (4000mg)	2	44.0000
Sig.		.083

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

RBC (x1012 cells/L)**

Duncan[a]

Sample	N	Subset for alpha = .05
		1
Group II (4000mg)	2	7.4250
Control	2	7.6150
Group I (2000mg)	2	7.7750
Group III (6000mg)	2	8.5150
Sig.		.377

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

WBC (x1012 cells/L)**

Duncan[a]

Sample	N	Subset for alpha = .05
		1
Control	2	7.4500
Group I (2000mg)	2	7.5000
Group II (4000mg)	2	7.7500
Group III (6000mg)	2	8.2000
Sig.		.288

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Oneway (Day 14)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Body weight (g)	Control	2	178.9850	3.16077	2.23500	150.5866	207.3834	176.75	181.22
	Group I (2000mg)	2	180.3400	6.90136	4.88000	118.3337	242.3463	175.46	185.22
	Group II (4000mg)	2	199.5050	.96874	.68500	190.8012	208.2088	198.82	200.19
	Group III (6000mg)	2	188.5800	6.32153	4.47000	131.7833	245.3767	184.11	193.05
	Total	8	186.8525	9.51150	3.36282	178.9007	194.8043	175.46	200.19
PVC (%)	Control	2	43.5000	.70711	.50000	37.1469	49.8531	43.00	44.00
	Group I (2000mg)	2	43.0000	.00000	.00000	43.0000	43.0000	43.00	43.00
	Group II (4000mg)	2	38.5000	.70711	.50000	32.1469	44.8531	38.00	39.00
	Group III (6000mg)	2	43.0000	1.41421	1.00000	30.2938	55.7062	42.00	44.00
	Total	8	42.0000	2.26779	.80178	40.1041	43.8959	38.00	44.00
RBC (x10**12 cells/L)	Control	2	7.5250	.03536	.02500	7.2073	7.8427	7.50	7.55
	Group I (2000mg)	2	6.9100	1.00409	.71000	-2.1114	15.9314	6.20	7.62
	Group II (4000mg)	2	6.9300	.05657	.04000	6.4218	7.4382	6.89	6.97
	Group III (6000mg)	2	7.5800	.14142	.10000	6.3094	8.8506	7.48	7.68
	Total	8	7.2363	.51216	.18108	6.8081	7.6644	6.20	7.68
WBC (x10**12 cells/L)	Control	2	7.9500	.35355	.25000	4.7734	11.1266	7.70	8.20
	Group I (2000mg)	2	7.1500	.91924	.65000	-1.1090	15.4090	6.50	7.80
	Group II (4000mg)	2	7.5000	.42426	.30000	3.6881	11.3119	7.20	7.80
	Group III (6000mg)	2	7.6100	1.00409	.71000	-1.4114	16.6314	6.90	8.32
	Total	8	7.5525	.63362	.22402	7.0228	8.0822	6.50	8.32

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Body weight (g)	Between Groups	534.760	3	178.253	7.237	.043
	Within Groups	98.519	4	24.630		
	Total	633.280	7			
PVC (%)	Between Groups	33.000	3	11.000	14.667	.013
	Within Groups	3.000	4	.750		
	Total	36.000	7			
RBC (x10**12 cells/L)	Between Groups	.804	3	.268	1.038	.466
	Within Groups	1.033	4	.258		
	Total	1.836	7			
WBC (x10**12 cells/L)	Between Groups	.652	3	.217	.403	.759
	Within Groups	2.158	4	.540		
	Total	2.810	7			

Post Hoc Tests

Homogeneous Subsets

Body weight (g)

Duncan[a]

		Subset for alpha = .05	
Sample	N	1	2
Control	2	178.9850	
Group I (2000mg)	2	180.3400	
Group III (6000mg)	2	188.5800	188.5800
Group II (4000mg)	2		199.5050
Sig.		.130	.093

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

PVC (%)

Duncan[a]

Sample	N	Subset for alpha = .05	
		1	2
Group II (4000mg)	2	38.5000	
Group I (2000mg)	2		43.0000
Group III (6000mg)	2		43.0000
Control	2		43.5000
Sig.		1.000	.599

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Oneway (Day 21)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Body weight (g)	Control	2	179.0400	1.97990	1.40000	161.2513	196.8287	177.64	180.44
	Group I (2000mg)	2	180.2400	6.26497	4.43000	123.9515	236.5285	175.81	184.67
	Group II (4000mg)	2	197.4650	2.04354	1.44500	179.1045	215.8255	196.02	198.91
	Group III (6000mg)	2	188.5300	7.09935	5.02000	124.7449	252.3151	183.51	193.55
	Total	8	186.3188	8.74942	3.09339	179.0041	193.6334	175.81	198.91
PVC (%)	Control	2	42.0000	2.82843	2.00000	16.5876	67.4124	40.00	44.00
	Group I (2000mg)	2	42.0000	1.41421	1.00000	29.2938	54.7062	41.00	43.00
	Group II (4000mg)	2	46.5000	4.94975	3.50000	2.0283	90.9717	43.00	50.00
	Group III (6000mg)	2	42.5000	.70711	.50000	36.1469	48.8531	42.00	43.00
	Total	8	43.2500	3.01188	1.06486	40.7320	45.7680	40.00	50.00
RBC (x10**12 cells/L)	Control	2	7.9900	.66468	.47000	2.0181	13.9619	7.52	8.46
	Group I (2000mg)	2	8.1800	.60811	.43000	2.7163	13.6437	7.75	8.61
	Group II (4000mg)	2	8.2650	.06364	.04500	7.6932	8.8368	8.22	8.31
	Group III (6000mg)	2	9.0150	.16263	.11500	7.5538	10.4762	8.90	9.13
	Total	8	8.3625	.54205	.19164	7.9093	8.8157	7.52	9.13
WBC (x10**12 cells/L)	Control	2	7.9000	.14142	.10000	6.6294	9.1706	7.80	8.00
	Group I (2000mg)	2	7.2500	1.20208	.85000	-3.5503	18.0503	6.40	8.10
	Group II (4000mg)	2	7.5000	.42426	.30000	3.6881	11.3119	7.20	7.80
	Group III (6000mg)	2	7.9500	1.06066	.75000	-1.5797	17.4797	7.20	8.70
	Total	8	7.6500	.70102	.24785	7.0639	8.2361	6.40	8.70

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Body weight (g)	Between Groups	438.120	3	146.040	5.976	.058
	Within Groups	97.747	4	24.437		
	Total	535.866	7			
PVC (%)	Between Groups	28.500	3	9.500	1.086	.451
	Within Groups	35.000	4	8.750		
	Total	63.500	7			
RBC (x10**12 cells/L)	Between Groups	1.215	3	.405	1.923	.267
	Within Groups	.842	4	.211		
	Total	2.057	7			
WBC (x10**12 cells/L)	Between Groups	.670	3	.223	.323	.810
	Within Groups	2.770	4	.692		
	Total	3.440	7			

Post Hoc Tests

Homogeneous Subsets

Body weight (g)

Duncan[a]

Sample	N	Subset for alpha = .05	
		1	2
Control	2	179.0400	
Group I (2000mg)	2	180.2400	
Group III (6000mg)	2	188.5300	188.5300
Group II (4000mg)	2		197.4650
Sig.		.132	.145

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

PVC (%)

Duncan[a]

Sample	N	Subset for alpha = .05
		1
Control	2	42.0000
Group I (2000mg)	2	42.0000
Group III (6000mg)	2	42.5000
Group II (4000mg)	2	46.5000
Sig.		.208

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

RBC (x1012 cells/L)**

Duncan[a]

Sample	N	Subset for alpha = .05
		1
Control	2	7.9900
Group I (2000mg)	2	8.1800
Group II (4000mg)	2	8.2650
Group III (6000mg)	2	9.0150
Sig.		.094

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

WBC (x1012 cells/L)**

Duncan[a]

Sample	N	Subset for alpha = .05
		1
Group I (2000mg)	2	7.2500
Group II (4000mg)	2	7.5000
Control	2	7.9000
Group III (6000mg)	2	7.9500
Sig.		.450

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Oneway (Day 28)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Body weight (g)	Control	2	178.8550	2.73650	1.93500	154.2685	203.4415	176.92	180.79
	Group I (2000mg)	2	179.9250	4.82954	3.41500	136.5333	223.3167	176.51	183.34
	Group II (4000mg)	2	197.0900	2.63044	1.86000	173.4565	220.7235	195.23	198.95
	Group III (6000mg	2	188.0050	7.03571	4.97500	124.7916	251.2184	183.03	192.98
	Total	8	185.9688	8.59531	3.03890	178.7829	193.1546	176.51	198.95
PVC (%)	Control	2	44.0000	2.82843	2.00000	18.5876	69.4124	42.00	46.00
	Group I (2000mg)	2	43.5000	2.12132	1.50000	24.4407	62.5593	42.00	45.00
	Group II (4000mg)	2	44.0000	.00000	.00000	44.0000	44.0000	44.00	44.00
	Group III (6000mg	2	41.0000	1.41421	1.00000	28.2938	53.7062	40.00	42.00
	Total	8	43.1250	1.95941	.69276	41.4869	44.7631	40.00	46.00
RBC (x10**12 cells/L)	Control	2	8.0150	.30406	.21500	5.2832	10.7468	7.80	8.23
	Group I (2000mg)	2	7.7000	.18385	.13000	6.0482	9.3518	7.57	7.83
	Group II (4000mg)	2	8.0400	.50912	.36000	3.4658	12.6142	7.68	8.40
	Group III (6000mg	2	7.9600	.62225	.44000	2.3693	13.5507	7.52	8.40
	Total	8	7.9288	.36231	.12810	7.6258	8.2317	7.52	8.40
WBC (x10**12 cells/L	Control	2	8.2000	.42426	.30000	4.3881	12.0119	7.90	8.50
	Group I (2000mg)	2	7.3500	.77782	.55000	.3616	14.3384	6.80	7.90
	Group II (4000mg)	2	7.8750	.17678	.12500	6.2867	9.4633	7.75	8.00
	Group III (6000mg	2	8.2750	.45962	.32500	4.1455	12.4045	7.95	8.60
	Total	8	7.9250	.54642	.19319	7.4682	8.3818	6.80	8.60

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Body weight (g)	Between Groups	429.922	3	143.307	6.571	.050
	Within Groups	87.233	4	21.808		
	Total	517.155	7			
PVC (%)	Between Groups	12.375	3	4.125	1.138	.435
	Within Groups	14.500	4	3.625		
	Total	26.875	7			
RBC (x10**12 cells/L)	Between Groups	.146	3	.049	.252	.856
	Within Groups	.773	4	.193		
	Total	.919	7			
WBC (x10**12 cells/L)	Between Groups	1.063	3	.354	1.379	.370
	Within Groups	1.028	4	.257		
	Total	2.090	7			

Post Hoc Tests

Homogeneous Subsets

Body weight (g)

Duncan[a]

Sample	N	Subset for alpha = .05	
		1	2
Control	2	178.8550	
Group I (2000mg)	2	179.9250	
Group III (6000mg)	2	188.0050	188.0050
Group II (4000mg)	2		197.0900
Sig.		.127	.124

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Oneway (Day 35)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Body weight (g)	Control	2	178.0150	4.24971	3.00500	139.8329	216.1971	175.01	181.02
	Group I (2000mg)	2	178.9350	4.27800	3.02500	140.4987	217.3713	175.91	181.96
	Group II (4000mg)	2	195.2700	5.30330	3.75000	147.6217	242.9183	191.52	199.02
	Group III (6000mg)	2	190.4850	9.15703	6.47500	108.2123	272.7577	184.01	196.96
	Total	8	185.6763	9.15692	3.23746	178.0209	193.3316	175.01	199.02
PVC (%)	Control	2	44.5000	.70711	.50000	38.1469	50.8531	44.00	45.00
	Group I (2000mg)	2	43.0000	1.41421	1.00000	30.2938	55.7062	42.00	44.00
	Group II (4000mg)	2	43.0000	.00000	.00000	43.0000	43.0000	43.00	43.00
	Group III (6000mg)	2	41.0000	.00000	.00000	41.0000	41.0000	41.00	41.00
	Total	8	42.8750	1.45774	.51539	41.6563	44.0937	41.00	45.00
RBC (x10**12 cells/L)	Control	2	8.4450	.34648	.24500	5.3320	11.5580	8.20	8.69
	Group I (2000mg)	2	8.5500	.07071	.05000	7.9147	9.1853	8.50	8.60
	Group II (4000mg)	2	8.2350	.13435	.09500	7.0279	9.4421	8.14	8.33
	Group III (6000mg)	2	8.6000	.70711	.50000	2.2469	14.9531	8.10	9.10
	Total	8	8.4575	.33809	.11953	8.1748	8.7402	8.10	9.10
WBC (x10**12 cells/L)	Control	2	7.9000	.70711	.50000	1.5469	14.2531	7.40	8.40
	Group I (2000mg)	2	7.7750	.17678	.12500	6.1867	9.3633	7.65	7.90
	Group II (4000mg)	2	8.0000	.28284	.20000	5.4588	10.5412	7.80	8.20
	Group III (6000mg)	2	8.1000	.56569	.40000	3.0175	13.1825	7.70	8.50
	Total	8	7.9438	.38678	.13675	7.6204	8.2671	7.40	8.50

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Body weight (g)	Between Groups	438.607	3	146.202	3.942	.109
	Within Groups	148.338	4	37.084		
	Total	586.944	7			
PVC (%)	Between Groups	12.375	3	4.125	6.600	.050
	Within Groups	2.500	4	.625		
	Total	14.875	7			
RBC (x10**12 cells/L)	Between Groups	.157	3	.052	.326	.808
	Within Groups	.643	4	.161		
	Total	.800	7			
WBC (x10**12 cells/L)	Between Groups	.116	3	.039	.166	.914
	Within Groups	.931	4	.233		
	Total	1.047	7			

Post Hoc Tests

Homogeneous Subsets

Body weight (g)

Duncan[a]

Sample	N	Subset for alpha = .05
		1
Control	2	178.0150
Group I (2000mg)	2	178.9350
Group III (6000mg)	2	190.4850
Group II (4000mg)	2	195.2700
Sig.		.051

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

PVC (%)

Duncan[a]

Sample	N	Subset for alpha = .05	
		1	2
Group III (6000mg)	2	41.0000	
Group I (2000mg)	2	43.0000	43.0000
Group II (4000mg)	2	43.0000	43.0000
Control	2		44.5000
Sig.		.068	.136

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

RBC (x1012 cells/L)**

Duncan[a]

Sample	N	Subset for alpha = .05
		1
Group II (4000mg)	2	8.2350
Control	2	8.4450
Group I (2000mg)	2	8.5500
Group III (6000mg)	2	8.6000
Sig.		.417

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

WBC (x1012 cells/L)**

Duncan[a]

Sample	N	Subset for alpha = .05
		1
Group I (2000mg)	2	7.7750
Control	2	7.9000
Group II (4000mg)	2	8.0000
Group III (6000mg)	2	8.1000
Sig.		.539

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Oneway (Day 42)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Body weight (g)	Control	2	176.5850	4.29214	3.03500	138.0217	215.1483	173.55	179.62
	Group I (2000mg)	2	178.3950	1.36472	.96500	166.1335	190.6565	177.43	179.36
	Group II (4000mg)	2	194.8600	9.37624	6.63000	110.6179	279.1021	188.23	201.49
	Group III (6000mg)	2	188.2600	9.46109	6.69000	103.2555	273.2645	181.57	194.95
	Total	8	184.5250	9.56539	3.38188	176.5281	192.5219	173.55	201.49
PVC (%)	Control	2	46.5000	.70711	.50000	40.1469	52.8531	46.00	47.00
	Group I (2000mg)	2	46.0000	2.82843	2.00000	20.5876	71.4124	44.00	48.00
	Group II (4000mg)	2	45.0000	1.41421	1.00000	32.2938	57.7062	44.00	46.00
	Group III (6000mg)	2	44.5000	.70711	.50000	38.1469	50.8531	44.00	45.00
	Total	8	45.5000	1.51186	.53452	44.2361	46.7639	44.00	48.00
RBC (x10**12 cells/L)	Control	2	8.6000	.33941	.24000	5.5505	11.6495	8.36	8.84
	Group I (2000mg)	2	9.0800	.08485	.06000	8.3176	9.8424	9.02	9.14
	Group II (4000mg)	2	9.2450	.07778	.05500	8.5462	9.9438	9.19	9.30
	Group III (6000mg)	2	9.2100	.28284	.20000	6.6688	11.7512	9.01	9.41
	Total	8	9.0338	.32522	.11498	8.7619	9.3056	8.36	9.41
WBC (x10**12 cells/L)	Control	2	7.8250	1.16673	.82500	-2.6576	18.3076	7.00	8.65
	Group I (2000mg)	2	8.1750	.45962	.32500	4.0455	12.3045	7.85	8.50
	Group II (4000mg)	2	8.7250	1.59099	1.12500	-5.5695	23.0195	7.60	9.85
	Group III (6000mg)	2	7.5000	.42426	.30000	3.6881	11.3119	7.20	7.80
	Total	8	8.0563	.92057	.32547	7.2866	8.8259	7.00	9.85

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Body weight (g)	Between Groups	442.766	3	147.589	2.986	.159
	Within Groups	197.711	4	49.428		
	Total	640.477	7			
PVC (%)	Between Groups	5.000	3	1.667	.606	.645
	Within Groups	11.000	4	2.750		
	Total	16.000	7			
RBC (x10**12 cells/L)	Between Groups	.532	3	.177	3.402	.134
	Within Groups	.208	4	.052		
	Total	.740	7			
WBC (x10**12 cells/L)	Between Groups	1.648	3	.549	.513	.695
	Within Groups	4.284	4	1.071		
	Total	5.932	7			

Oneway (Control)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Body weight (g)	0	2	177.2400	3.57796	2.53000	145.0933	209.3867	174.71	179.77
	7	2	178.3000	.53740	.38000	173.4716	183.1284	177.92	178.68
	14	2	178.9850	3.16077	2.23500	150.5866	207.3834	176.75	181.22
	21	2	179.0400	1.97990	1.40000	161.2513	196.8287	177.64	180.44
	28	2	178.8550	2.73650	1.93500	154.2685	203.4415	176.92	180.79
	35	2	178.0150	4.24971	3.00500	139.8329	216.1971	175.01	181.02
	42	2	176.5850	4.29214	3.03500	138.0217	215.1483	173.55	179.62
	Total	14	178.1457	2.50508	.66951	176.6993	179.5921	173.55	181.22
PVC (%)	0	2	41.5000	.70711	.50000	35.1469	47.8531	41.00	42.00
	7	2	41.0000	1.41421	1.00000	28.2938	53.7062	40.00	42.00
	14	2	43.5000	.70711	.50000	37.1469	49.8531	43.00	44.00
	21	2	42.0000	2.82843	2.00000	16.5876	67.4124	40.00	44.00
	28	2	44.0000	2.82843	2.00000	18.5876	69.4124	42.00	46.00
	35	2	44.5000	.70711	.50000	38.1469	50.8531	44.00	45.00
	42	2	46.5000	.70711	.50000	40.1469	52.8531	46.00	47.00
	Total	14	43.2857	2.23361	.59696	41.9961	44.5754	40.00	47.00
RBC (x10**12 cells/L)	0	2	7.6500	.49497	.35000	3.2028	12.0972	7.30	8.00
	7	2	7.6150	1.25158	.88500	-3.6300	18.8600	6.73	8.50
	14	2	7.5250	.03536	.02500	7.2073	7.8427	7.50	7.55
	21	2	7.9900	.66468	.47000	2.0181	13.9619	7.52	8.46
	28	2	8.0150	.30406	.21500	5.2832	10.7468	7.80	8.23
	35	2	8.4450	.34648	.24500	5.3320	11.5580	8.20	8.69
	42	2	8.6000	.33941	.24000	5.5505	11.6495	8.36	8.84
	Total	14	7.9771	.60027	.16043	7.6306	8.3237	6.73	8.84
WBC (x10**12 cells/L)	0	2	7.4000	.28284	.20000	4.8588	9.9412	7.20	7.60
	7	2	7.4500	.63640	.45000	1.7322	13.1678	7.00	7.90
	14	2	7.9500	.35355	.25000	4.7734	11.1266	7.70	8.20
	21	2	7.9000	.14142	.10000	6.6294	9.1706	7.80	8.00
	28	2	8.2000	.42426	.30000	4.3881	12.0119	7.90	8.50
	35	2	7.9000	.70711	.50000	1.5469	14.2531	7.40	8.40
	42	2	7.8250	1.16673	.82500	-2.6576	18.3076	7.00	8.65
	Total	14	7.8036	.52932	.14147	7.4980	8.1092	7.00	8.65

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Body weight (g)	Between Groups	10.609	6	1.768	.174	.975
	Within Groups	70.972	7	10.139		
	Total	81.581	13			
PVC (%)	Between Groups	44.857	6	7.476	2.617	.117
	Within Groups	20.000	7	2.857		
	Total	64.857	13			
RBC (x10**12 cells/L)	Between Groups	2.102	6	.350	.950	.517
	Within Groups	2.582	7	.369		
	Total	4.684	13			
WBC (x10**12 cells/L)	Between Groups	.971	6	.162	.424	.842
	Within Groups	2.671	7	.382		
	Total	3.642	13			

Post Hoc Tests

Homogeneous Subsets

PVC (%)

Duncan[a]

Days of Treatment	N	Subset for alpha = .05	
		1	2
7	2	41.0000	
0	2	41.5000	
21	2	42.0000	
14	2	43.5000	43.5000
28	2	44.0000	44.0000
35	2	44.5000	44.5000
42	2		46.5000
Sig.		.095	.137

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Oneway (Group I)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Body weight (g)	0	2	179.1400	2.14960	1.52000	159.8266	198.4534	177.62	180.66
	7	2	179.2950	7.03571	4.97500	116.0816	242.5084	174.32	184.27
	14	2	180.3400	6.90136	4.88000	118.3337	242.3463	175.46	185.22
	21	2	180.2400	6.26497	4.43000	123.9515	236.5285	175.81	184.67
	28	2	179.9250	4.82954	3.41500	136.5333	223.3167	176.51	183.34
	35	2	178.9350	4.27800	3.02500	140.4987	217.3713	175.91	181.96
	42	2	178.3950	1.36472	.96500	166.1335	190.6565	177.43	179.36
	Total	14	179.4671	3.83070	1.02380	177.2554	181.6789	174.32	185.22
PVC (%)	0	2	43.5000	.70711	.50000	37.1469	49.8531	43.00	44.00
	7	2	43.0000	.00000	.00000	43.0000	43.0000	43.00	43.00
	14	2	43.0000	.00000	.00000	43.0000	43.0000	43.00	43.00
	21	2	42.0000	1.41421	1.00000	29.2938	54.7062	41.00	43.00
	28	2	43.5000	2.12132	1.50000	24.4407	62.5593	42.00	45.00
	35	2	43.0000	1.41421	1.00000	30.2938	55.7062	42.00	44.00
	42	2	46.0000	2.82843	2.00000	20.5876	71.4124	44.00	48.00
	Total	14	43.4286	1.65084	.44121	42.4754	44.3817	41.00	48.00
RBC (x10**12 cells/L)	0	2	7.8050	.48790	.34500	3.4214	12.1886	7.46	8.15
	7	2	7.7750	1.61927	1.14500	-6.7736	22.3236	6.63	8.92
	14	2	6.9100	1.00409	.71000	-2.1114	15.9314	6.20	7.62
	21	2	8.1800	.60811	.43000	2.7163	13.6437	7.75	8.61
	28	2	7.7000	.18385	.13000	6.0482	9.3518	7.57	7.83
	35	2	8.5500	.07071	.05000	7.9147	9.1853	8.50	8.60
	42	2	9.0800	.08485	.06000	8.3176	9.8424	9.02	9.14
	Total	14	8.0000	.87794	.23464	7.4931	8.5069	6.20	9.14
WBC (x10**12 cells/L	0	2	7.0500	.77782	.55000	.0616	14.0384	6.50	7.60
	7	2	7.5000	.70711	.50000	1.1469	13.8531	7.00	8.00
	14	2	7.1500	.91924	.65000	-1.1090	15.4090	6.50	7.80
	21	2	7.2500	1.20208	.85000	-3.5503	18.0503	6.40	8.10
	28	2	7.3500	.77782	.55000	.3616	14.3384	6.80	7.90
	35	2	7.7750	.17678	.12500	6.1867	9.3633	7.65	7.90
	42	2	8.1750	.45962	.32500	4.0455	12.3045	7.85	8.50
	Total	14	7.4643	.68541	.18318	7.0685	7.8600	6.40	8.50

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Body weight (g)	Between Groups	6.276	6	1.046	.040	1.000
	Within Groups	184.489	7	26.356		
	Total	190.765	13			
PVC (%)	Between Groups	18.429	6	3.071	1.265	.379
	Within Groups	17.000	7	2.429		
	Total	35.429	13			
RBC (x10**12 cells/L)	Between Groups	5.736	6	.956	1.562	.285
	Within Groups	4.284	7	.612		
	Total	10.020	13			
WBC (x10**12 cells/L)	Between Groups	1.865	6	.311	.513	.783
	Within Groups	4.242	7	.606		
	Total	6.107	13			

Post Hoc Tests

Homogeneous Subsets

Body weight (g)

Duncan[a]

Days of Treatment	N	Subset for alpha = .05
		1
42	2	178.3950
35	2	178.9350
0	2	179.1400
7	2	179.2950
28	2	179.9250
21	2	180.2400
14	2	180.3400
Sig.		.725

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

PVC (%)

Duncan[a]

Days of Treatment	N	Subset for alpha = .05	
		1	2
21	2	42.0000	
7	2	43.0000	43.0000
14	2	43.0000	43.0000
35	2	43.0000	43.0000
0	2	43.5000	43.5000
28	2	43.5000	43.5000
42	2		46.0000
Sig.		.391	.115

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

RBC (x1012 cells/L)**

Duncan[a]

Days of Treatment	N	Subset for alpha = .05	
		1	2
14	2	6.9100	
28	2	7.7000	7.7000
7	2	7.7750	7.7750
0	2	7.8050	7.8050
21	2	8.1800	8.1800
35	2	8.5500	8.5500
42	2		9.0800
Sig.		.092	.143

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

WBC (x1012 cells/L)**

Duncan[a]

Days of Treatment	N	Subset for alpha = .05
		1
0	2	7.0500
14	2	7.1500
21	2	7.2500
28	2	7.3500
7	2	7.5000
35	2	7.7750
42	2	8.1750
Sig.		.215

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Oneway (Group II)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Body weight (g)	0	2	198.9400	2.37588	1.68000	177.5936	220.2864	197.26	200.62
	7	2	200.7700	2.48902	1.76000	178.4071	223.1329	199.01	202.53
	14	2	199.5050	.96874	.68500	190.8012	208.2088	198.82	200.19
	21	2	197.4650	2.04354	1.44500	179.1045	215.8255	196.02	198.91
	28	2	197.0900	2.63044	1.86000	173.4565	220.7235	195.23	198.95
	35	2	195.2700	5.30330	3.75000	147.6217	242.9183	191.52	199.02
	42	2	194.8600	9.37624	6.63000	110.6179	279.1021	188.23	201.49
	Total	14	197.7000	3.89427	1.04079	195.4515	199.9485	188.23	202.53
PVC (%)	0	2	42.5000	.70711	.50000	36.1469	48.8531	42.00	43.00
	7	2	44.0000	.00000	.00000	44.0000	44.0000	44.00	44.00
	14	2	38.5000	.70711	.50000	32.1469	44.8531	38.00	39.00
	21	2	46.5000	4.94975	3.50000	2.0283	90.9717	43.00	50.00
	28	2	44.0000	.00000	.00000	44.0000	44.0000	44.00	44.00
	35	2	43.0000	.00000	.00000	43.0000	43.0000	43.00	43.00
	42	2	45.0000	1.41421	1.00000	32.2938	57.7062	44.00	46.00
	Total	14	43.3571	2.81772	.75307	41.7302	44.9840	38.00	50.00
RBC (x10**12 cells/L)	0	2	8.0900	.19799	.14000	6.3111	9.8689	7.95	8.23
	7	2	7.4250	.74246	.52500	.7542	14.0958	6.90	7.95
	14	2	6.9300	.05657	.04000	6.4218	7.4382	6.89	6.97
	21	2	8.2650	.06364	.04500	7.6932	8.8368	8.22	8.31
	28	2	8.0400	.50912	.36000	3.4658	12.6142	7.68	8.40
	35	2	8.2350	.13435	.09500	7.0279	9.4421	8.14	8.33
	42	2	9.2450	.07778	.05500	8.5462	9 9438	9.19	9.30
	Total	14	8.0329	.74355	.19872	7.6035	8.4622	6.89	9.30
WBC (x10**12 cells/L)	0	2	7.3500	.49497	.35000	2.9028	11.7972	7.00	7.70
	7	2	7.7500	.49497	.35000	3.3028	12.1972	7.40	8.10
	14	2	7.5000	.42426	.30000	3.6881	11.3119	7.20	7.80
	21	2	7.5000	.42426	.30000	3.6881	11.3119	7.20	7.80
	28	2	7.8750	.17678	.12500	6.2867	9.4633	7.75	8.00
	35	2	8.0000	.28284	.20000	5.4588	10.5412	7.80	8.20
	42	2	8.7250	1.59099	1.12500	-5.5695	23.0195	7.60	9.85
	Total	14	7.8143	.68288	.18251	7.4200	8.2086	7.00	9.85

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Body weight (g)	Between Groups	57.237	6	9.539	.477	.807
	Within Groups	139.913	7	19.988		
	Total	197.149	13			
PVC (%)	Between Groups	75.714	6	12.619	3.212	.076
	Within Groups	27.500	7	3.929		
	Total	103.214	13			
RBC (x10**12 cells/L)	Between Groups	6.306	6	1.051	8.351	.007
	Within Groups	.881	7	.126		
	Total	7.187	13			
WBC (x10**12 cells/L)	Between Groups	2.570	6	.428	.858	.566
	Within Groups	3.492	7	.499		
	Total	6.062	13			

Post Hoc Tests

Homogeneous Subsets

PVC (%)

Duncan[a]

Days of Treatment	N	Subset for alpha = .05	
		1	2
14	2	38.5000	
0	2	42.5000	42.5000
35	2	43.0000	43.0000
7	2		44.0000
28	2		44.0000
42	2		45.0000
21	2		46.5000
Sig.		.065	.102

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

RBC (x1012 cells/L)**

Duncan[a]

Days of Treatment	N	Subset for alpha = .05		
		1	2	3
14	2	6.9300		
7	2	7.4250	7.4250	
28	2		8.0400	
0	2		8.0900	
35	2		8.2350	
21	2		8.2650	
42	2			9.2450
Sig.		.206	.063	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Oneway (Group III)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Body weight (g)	0	2	194.1600	8.71156	6.16000	115.8898	272.4302	188.00	200.32
	7	2	190.6600	6.15183	4.35000	135.3880	245.9320	186.31	195.01
	14	2	188.5800	6.32153	4.47000	131.7833	245.3767	184.11	193.05
	21	2	188.5300	7.09935	5.02000	124.7449	252.3151	183.51	193.55
	28	2	188.0050	7.03571	4.97500	124.7916	251.2184	183.03	192.98
	35	2	190.4850	9.15703	6.47500	108.2123	272.7577	184.01	196.96
	42	2	188.2600	9.46109	6.69000	103.2555	273.2645	181.57	194.95
	Total	14	189.8114	6.10667	1.63208	186.2855	193.3373	181.57	200.32
PVC (%)	0	2	41.5000	.70711	.50000	35.1469	47.8531	41.00	42.00
	7	2	41.5000	2.12132	1.50000	22.4407	60.5593	40.00	43.00
	14	2	43.0000	1.41421	1.00000	30.2938	55.7062	42.00	44.00
	21	2	42.5000	.70711	.50000	36.1469	48.8531	42.00	43.00
	28	2	41.0000	1.41421	1.00000	28.2938	53.7062	40.00	42.00
	35	2	41.0000	.00000	.00000	41.0000	41.0000	41.00	41.00
	42	2	44.5000	.70711	.50000	38.1469	50.8531	44.00	45.00
	Total	14	42.1429	1.51186	.40406	41.2699	43.0158	40.00	45.00
RBC (x10**12 cells/L)	0	2	8.8200	.26870	.19000	6.4058	11.2342	8.63	9.01
	7	2	8.5150	.12021	.08500	7.4350	9.5950	8.43	8.60
	14	2	7.5800	.14142	.10000	6.3094	8.8506	7.48	7.68
	21	2	9.0150	.16263	.11500	7.5538	10.4762	8.90	9.13
	28	2	7.9600	.62225	.44000	2.3693	13.5507	7.52	8.40
	35	2	8.6000	.70711	.50000	2.2469	14.9531	8.10	9.10
	42	2	9.2100	.28284	.20000	6.6688	11.7512	9.01	9.41
	Total	14	8.5286	.62811	.16787	8.1659	8.8912	7.48	9.41
WBC (x10**12 cells/L)	0	2	7.9000	.56569	.40000	2.8175	12.9825	7.50	8.30
	7	2	8.2000	.56569	.40000	3.1175	13.2825	7.80	8.60
	14	2	7.6100	1.00409	.71000	-1.4114	16.6314	6.90	8.32
	21	2	7.9500	1.06066	.75000	-1.5797	17.4797	7.20	8.70
	28	2	8.2750	.45962	.32500	4.1455	12.4045	7.95	8.60
	35	2	8.1000	.56569	.40000	3.0175	13.1825	7.70	8.50
	42	2	7.5000	.42426	.30000	3.6881	11.3119	7.20	7.80
	Total	14	7.9336	.58855	.15730	7.5938	8.2734	6.90	8.70

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Body weight (g)	Between Groups	57.825	6	9.637	.158	.981
	Within Groups	426.964	7	60.995		
	Total	484.788	13			
PVC (%)	Between Groups	19.714	6	3.286	2.300	.150
	Within Groups	10.000	7	1.429		
	Total	29.714	13			
RBC (x10**12 cells/L)	Between Groups	4.028	6	.671	4.271	.039
	Within Groups	1.100	7	.157		
	Total	5.129	13			
WBC (x10**12 cells/L)	Between Groups	1.019	6	.170	.341	.894
	Within Groups	3.484	7	.498		
	Total	4.503	13			

Post Hoc Tests

Homogeneous Subsets

Body weight (g)

Duncan[a]

Days of Treatment	N	Subset for alpha = .05
		1
28	2	188.0050
42	2	188.2600
21	2	188.5300
14	2	188.5800
35	2	190.4850
7	2	190.6600
0	2	194.1600
Sig.		.475

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

PVC (%)

Duncan[a]

Days of Treatment	N	Subset for alpha = .05	
		1	2
28	2	41.0000	
35	2	41.0000	
0	2	41.5000	41.5000
7	2	41.5000	41.5000
21	2	42.5000	42.5000
14	2	43.0000	43.0000
42	2		44.5000
Sig.		.161	.052

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

RBC (x1012 cells/L)**

Duncan[a]

Days of Treatment	N	Subset for alpha = .05		
		1	2	3
14	2	7.5800		
28	2	7.9600	7.9600	
7	2	8.5150	8.5150	8.5150
35	2		8.6000	8.6000
0	2		8.8200	8.8200
21	2			9.0150
42	2			9.2100
Sig.		.058	.080	.144

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

WBC (x1012 cells/L)**

Duncan[a]

Days of Treatment	N	Subset for alpha = .05
		1
42	2	7.5000
14	2	7.6100
0	2	7.9000
21	2	7.9500
35	2	8.1000
7	2	8.2000
28	2	8.2750
Sig.		.331

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

General Linear Model

Between-Subjects Factors

		Value Label	N
Sample	1.00	Control	14
	2.00	Group I (2000mg)	14
	3.00	Group II (4000mg)	14
	4.00	Group III (6000mg)	14
Days of Treatment	.00	0	8
	1.00	7	8
	2.00	14	8
	3.00	21	8
	4.00	28	8
	5.00	35	8
	6.00	42	8

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Body weight (g)	3708.513[a]	27	137.352	4.677	.000
	PVC (%)	174.339[b]	27	6.457	2.427	.011
	RBC (x10**12 cells/L)	21.092[c]	27	.781	2.472	.010
	WBC (x10**12 cells/L)	8.136[d]	27	.301	.607	.901
Intercept	Body weight (g)	1943235.704	1	1943235.704	66165.833	.000
	PVC (%)	103802.161	1	103802.161	39012.893	.000
	RBC (x10**12 cells/L)	3705.655	1	3705.655	11727.287	.000
	WBC (x10**12 cells/L)	3366.911	1	3366.911	6786.807	.000
Sample2	Body weight (g)	3576.566	3	1192.189	40.593	.000
	PVC (%)	15.625	3	5.208	1.957	.143
	RBC (x10**12 cells/L)	2.919	3	.973	3.079	.044
	WBC (x10**12 cells/L)	1.712	3	.571	1.150	.346
Days	Body weight (g)	48.095	6	8.016	.273	.945
	PVC (%)	66.214	6	11.036	4.148	.004
	RBC (x10**12 cells/L)	15.258	6	2.543	8.048	.000
	WBC (x10**12 cells/L)	2.537	6	.423	.852	.541
Sample2 * Days	Body weight (g)	83.852	18	4.658	.159	1.000
	PVC (%)	92.500	18	5.139	1.931	.057
	RBC (x10**12 cells/L)	2.915	18	.162	.513	.929
	WBC (x10**12 cells/L)	3.887	18	.216	.435	.965
Error	Body weight (g)	822.337	28	29.369		
	PVC (%)	74.500	28	2.661		
	RBC (x10**12 cells/L)	8.848	28	.316		
	WBC (x10**12 cells/L)	13.891	28	.496		
Total	Body weight (g)	1947766.553	56			
	PVC (%)	104051.000	56			
	RBC (x10**12 cells/L)	3735.594	56			
	WBC (x10**12 cells/L)	3388.937	56			
Corrected Total	Body weight (g)	4530.849	55			
	PVC (%)	248.839	55			
	RBC (x10**12 cells/L)	29.939	55			
	WBC (x10**12 cells/L)	22.027	55			

a. R Squared = .819 (Adjusted R Squared = .643)

b. R Squared = .701 (Adjusted R Squared = .412)

c. R Squared = .704 (Adjusted R Squared = .420)

d. R Squared = .369 (Adjusted R Squared = -.239)

Estimated Marginal Means

Grand Mean

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Body weight (g)	186.281	.724	184.798	187.765
PVC (%)	43.054	.218	42.607	43.500
RBC (x10**12 cells/L)	8.135	.075	7.981	8.289
WBC (x10**12 cells/L)	7.754	.094	7.561	7.947

Post Hoc Tests

Sample

Homogeneous Subsets

Body weight (g)

Duncan[a,b,c]

Sample	N	Subset		
		1	2	3
Control	14	178.1457		
Group I (2000mg)	14	179.4671		
Group III (6000mg)	14		189.8114	
Group II (4000mg)	14			197.7000
Sig.		.524	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 29.369.

a. Uses Harmonic Mean Sample Size = 14.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

RBC (x1012 cells/L)**

Duncan[a,b,c]

Sample	N	Subset 1	Subset 2
Control	14	7.9771	
Group I (2000mg)	14	8.0000	
Group II (4000mg)	14	8.0329	
Group III (6000mg)	14		8.5286
Sig.		.807	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .316.

a. Uses Harmonic Mean Sample Size = 14.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Days of Treatment

Homogeneous Subsets

PVC (%)

Duncan[a,b,c]

Days of Treatment	N	Subset 1	Subset 2
14	8	42.0000	
0	8	42.2500	
7	8	42.3750	
35	8	42.8750	
28	8	43.1250	
21	8	43.2500	
42	8		45.5000
Sig.		.188	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 2.661.

a. Uses Harmonic Mean Sample Size = 8.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

RBC (x1012 cells/L)**

Duncan[a,b,c]

Days of Treatment	N	Subset 1	Subset 2	Subset 3
14	8	7.2363		
7	8		7.8325	
28	8		7.9288	
0	8		8.0913	
21	8		8.3625	
35	8		8.4575	
42	8			9.0338
Sig.		1.000	.054	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .316.

a. Uses Harmonic Mean Sample Size = 8.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Oneway (Control)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Effect of B. sapida extract on weight (g) of diabetic rats	.00	3	185.8667	1.46401	.84525	182.2299	189.5035	184.30	187.20
	1.00	3	193.4333	5.88586	3.39820	178.8120	208.0546	189.50	200.20
	2.00	3	182.4667	1.95533	1.12891	177.6093	187.3240	180.60	184.50
	3.00	3	185.3000	.91652	.52915	183.0233	187.5767	184.30	186.10
	4.00	3	182.1333	1.15036	.66416	179.2757	184.9910	181.00	183.30
	Total	15	185.8400	4.88990	1.26257	183.1321	188.5479	180.60	200.20
Effect of M. oleifera extract on weight (g) of diabetic rats	.00	3	182.4667	1.95533	1.12891	177.6093	187.3240	180.60	184.50
	1.00	3	185.6000	4.08534	2.35867	175.4515	195.7485	180.90	188.30
	2.00	3	186.0000	.51962	.30000	184.7092	187.2908	185.40	186.30
	3.00	3	179.7000	1.57162	.90738	175.7959	183.6041	178.30	181.40
	4.00	3	175.9333	3.21455	1.85592	167.9479	183.9187	173.60	179.60
	Total	15	181.9400	4.47960	1.15663	179.4593	184.4207	173.60	188.30

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Effect of B. sapida extract on weight (g) of diabetic rats	Between Groups	249.209	4	62.302	7.283	.005
	Within Groups	85.547	10	8.555		
	Total	334.756	14			
Effect of M. oleifera extract on weight (g) of diabetic rats	Between Groups	213.763	4	53.441	7.956	.004
	Within Groups	67.173	10	6.717		
	Total	280.936	14			

Post Hoc Tests
Homogeneous Subsets

:ffect of B. sapida extract on weight (g) of diabetic rats

	Week	N	Subset for alpha = .05	
			1	2
Duncan[a]	4.00	3	182.1333	
	2.00	3	182.4667	
	3.00	3	185.3000	
	.00	3	185.8667	
	1.00	3		193.4333
	Sig.		.175	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Effect of M. oleifera extract on weight (g) of diabetic rats

	Week	N	Subset for alpha = .05		
			1	2	3
Duncan[a]	4.00	3	175.9333		
	3.00	3	179.7000	179.7000	
	.00	3		182.4667	182.4667
	1.00	3			185.6000
	2.00	3			186.0000
	Sig.		.105	.220	.142

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Multiple Comparisons

Dependent Variable		(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Effect of B. sapida extract on weight (g) of diabetic rats	LSD	.00	1.00	-7.56667*	2.38812	.010	-12.8877	-2.2456
			2.00	3.40000	2.38812	.185	-1.9211	8.7211
			3.00	.56667	2.38812	.817	-4.7544	5.8877
			4.00	3.73333	2.38812	.149	-1.5877	9.0544
		1.00	.00	7.56667*	2.38812	.010	2.2456	12.8877
			2.00	10.96667*	2.38812	.001	5.6456	16.2877
			3.00	8.13333*	2.38812	.007	2.8123	13.4544
			4.00	11.30000*	2.38812	.001	5.9789	16.6211
		2.00	.00	-3.40000	2.38812	.185	-8.7211	1.9211
			1.00	-10.96667*	2.38812	.001	-16.2877	-5.6456
			3.00	-2.83333	2.38812	.263	-8.1544	2.4877
			4.00	.33333	2.38812	.892	-4.9877	5.6544
		3.00	.00	-.56667	2.38812	.817	-5.8877	4.7544
			1.00	-8.13333*	2.38812	.007	-13.4544	-2.8123
			2.00	2.83333	2.38812	.263	-2.4877	8.1544
			4.00	3.16667	2.38812	.214	-2.1544	8.4877
		4.00	.00	-3.73333	2.38812	.149	-9.0544	1.5877
			1.00	-11.30000*	2.38812	.001	-16.6211	-5.9789
			2.00	-.33333	2.38812	.892	-5.6544	4.9877
			3.00	-3.16667	2.38812	.214	-8.4877	2.1544
Effect of M. oleifera extract on weight (g) of diabetic rats	LSD	.00	1.00	-3.13333	2.11618	.170	-7.8485	1.5818
			2.00	-3.53333	2.11618	.126	-8.2485	1.1818
			3.00	2.76667	2.11618	.220	-1.9485	7.4818
			4.00	6.53333*	2.11618	.011	1.8182	11.2485
		1.00	.00	3.13333	2.11618	.170	-1.5818	7.8485
			2.00	-.40000	2.11618	.854	-5.1151	4.3151
			3.00	5.90000*	2.11618	.019	1.1849	10.6151
			4.00	9.66667*	2.11618	.001	4.9515	14.3818
		2.00	.00	3.53333	2.11618	.126	-1.1818	8.2485
			1.00	.40000	2.11618	.854	-4.3151	5.1151
			3.00	6.30000*	2.11618	.014	1.5849	11.0151
			4.00	10.06667*	2.11618	.001	5.3515	14.7818
		3.00	.00	-2.76667	2.11618	.220	-7.4818	1.9485
			1.00	-5.90000*	2.11618	.019	-10.6151	-1.1849
			2.00	-6.30000*	2.11618	.014	-11.0151	-1.5849
			4.00	3.76667	2.11618	.105	-.9485	8.4818
		4.00	.00	-6.53333*	2.11618	.011	-11.2485	-1.8182
			1.00	-9.66667*	2.11618	.001	-14.3818	-4.9515
			2.00	-10.06667*	2.11618	.001	-14.7818	-5.3515
			3.00	-3.76667	2.11618	.105	-8.4818	.9485

*. The mean difference is significant at the .05 level.

Oneway (Treated)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Effect of B. sapida extract on weight (g) of diabetic rats	.00	3	186.9000	1.41067	.81445	183.3957	190.4043	185.60	188.40
	1.00	3	187.5333	1.26623	.73106	184.3878	190.6788	186.40	188.90
	2.00	3	191.4333	.85049	.49103	189.3206	193.5461	190.60	192.30
	3.00	3	198.6000	.91652	.52915	196.3233	200.8767	197.80	199.60
	4.00	3	197.8333	1.59478	.92075	193.8717	201.7950	196.50	199.60
	Total	15	192.4600	5.23611	1.35196	189.5603	195.3597	185.60	199.60
Effect of M. oleifera extract on weight (g) of diabetic rats	.00	3	181.6667	1.41892	.81921	178.1419	185.1915	180.40	183.20
	1.00	3	181.7667	5.64122	3.25696	167.7531	195.7802	176.80	187.90
	2.00	3	178.4333	1.15036	.66416	175.5757	181.2910	177.30	179.60
	3.00	3	179.6333	6.57597	3.79664	163.2977	195.9689	175.30	187.20
	4.00	3	177.6667	6.46091	3.73021	161.6169	193.7165	173.40	185.10
	Total	15	179.8333	4.48548	1.15815	177.3494	182.3173	173.40	187.90

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Effect of B. sapida extract on weight (g) of diabetic rats	Between Groups	368.436	4	92.109	59.811	.000
	Within Groups	15.400	10	1.540		
	Total	383.836	14			
Effect of M. oleifera extract on weight (g) of diabetic rats	Between Groups	41.380	4	10.345	.431	.784
	Within Groups	240.293	10	24.029		
	Total	281.673	14			

Post Hoc Tests
Homogeneous Subsets

Effect of B. sapida extract on weight (g) of diabetic rats

	Week	N	Subset for alpha = .05		
			1	2	3
Duncan[a]	.00	3	186.9000		
	1.00	3	187.5333		
	2.00	3		191.4333	
	4.00	3			197.8333
	3.00	3			198.6000
	Sig.		.546	1.000	.467

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Effect of M. oleifera extract on weight (g) of diabetic rats

	Week	N	Subset for alpha = .05
			1
Duncan [a]	4.00	3	177.6667
	2.00	3	178.4333
	3.00	3	179.6333
	.00	3	181.6667
	1.00	3	181.7667
	Sig.		.366

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Multiple Comparisons

Dependent Variable		(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Effect of B. sapida extract on weight (g) of diabetic rats	LSD	.00	1.00	-.63333	1.01325	.546	-2.8910	1.6243
			2.00	-4.53333*	1.01325	.001	-6.7910	-2.2757
			3.00	-11.70000*	1.01325	.000	-13.9577	-9.4423
			4.00	-10.93333*	1.01325	.000	-13.1910	-8.6757
		1.00	.00	.63333	1.01325	.546	-1.6243	2.8910
			2.00	-3.90000*	1.01325	.003	-6.1577	-1.6423
			3.00	-11.06667*	1.01325	.000	-13.3243	-8.8090
			4.00	-10.30000*	1.01325	.000	-12.5577	-8.0423
		2.00	.00	4.53333*	1.01325	.001	2.2757	6.7910
			1.00	3.90000*	1.01325	.003	1.6423	6.1577
			3.00	-7.16667*	1.01325	.000	-9.4243	-4.9090
			4.00	-6.40000*	1.01325	.000	-8.6577	-4.1423
		3.00	.00	11.70000*	1.01325	.000	9.4423	13.9577
			1.00	11.06667*	1.01325	.000	8.8090	13.3243
			2.00	7.16667*	1.01325	.000	4.9090	9.4243
			4.00	.76667	1.01325	.467	-1.4910	3.0243
		4.00	.00	10.93333*	1.01325	.000	8.6757	13.1910
			1.00	10.30000*	1.01325	.000	8.0423	12.5577
			2.00	6.40000*	1.01325	.000	4.1423	8.6577
			3.00	-.76667	1.01325	.467	-3.0243	1.4910
Effect of M. oleifera extract on weight (g) of diabetic rats	LSD	.00	1.00	-.10000	4.00244	.981	-9.0180	8.8180
			2.00	3.23333	4.00244	.438	-5.6847	12.1513
			3.00	2.03333	4.00244	.622	-6.8847	10.9513
			4.00	4.00000	4.00244	.341	-4.9180	12.9180
		1.00	.00	.10000	4.00244	.981	-8.8180	9.0180
			2.00	3.33333	4.00244	.424	-5.5847	12.2513
			3.00	2.13333	4.00244	.606	-6.7847	11.0513
			4.00	4.10000	4.00244	.330	-4.8180	13.0180
		2.00	.00	-3.23333	4.00244	.438	-12.1513	5.6847
			1.00	-3.33333	4.00244	.424	-12.2513	5.5847
			3.00	-1.20000	4.00244	.770	-10.1180	7.7180
			4.00	.76667	4.00244	.852	-8.1513	9.6847
		3.00	.00	-2.03333	4.00244	.622	-10.9513	6.8847
			1.00	-2.13333	4.00244	.606	-11.0513	6.7847
			2.00	1.20000	4.00244	.770	-7.7180	10.1180
			4.00	1.96667	4.00244	.634	-6.9513	10.8847
		4.00	.00	-4.00000	4.00244	.341	-12.9180	4.9180
			1.00	-4.10000	4.00244	.330	-13.0180	4.8180
			2.00	-.76667	4.00244	.852	-9.6847	8.1513
			3.00	-1.96667	4.00244	.634	-10.8847	6.9513

*. The mean difference is significant at the .05 level.

T-Test (Week 0)

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of B. sapida extract on weight (g) of diabetic rats	CONTROL Diabetic	3	185.8667	1.46401	.84525
	TREATED Diabetic	3	186.9000	1.41067	.81445
Effect of M. oleifera extract on weight (g) of diabetic rats	CONTROL Diabetic	3	182.4667	1.95533	1.12891
	TREATED Diabetic	3	181.6667	1.41892	.81921

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of B. sapida extract on weight (g) of diabetic rats	Equal variances assumed	.006	.942	-.880	4	.428	-1.03333	1.17379	-4.29229	2.22562
	Equal variances not assumed			-.880	3.995	.428	-1.03333	1.17379	-4.29406	2.22739
Effect of M. oleifera extract on weight (g) of diabetic rats	Equal variances assumed	.220	.663	.574	4	.597	.80000	1.39483	-3.07267	4.67267
	Equal variances not assumed			.574	3.649	.600	.80000	1.39483	-3.22407	4.82407

T-Test (Week 1)

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of B. sapida extract on weight (g) of diabetic rats	CONTROL Diabetic	3	193.4333	5.88586	3.39820
	TREATED Diabetic	3	187.5333	1.26623	.73106
Effect of M. oleifera extract on weight (g) of diabetic rats	CONTROL Diabetic	3	185.6000	4.08534	2.35867
	TREATED Diabetic	3	181.7667	5.64122	3.25696

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of B. sapida extract on weight (g) of diabetic rats	Equal variances assumed	8.686	.042	1.697	4	.165	5.90000	3.47595	-3.75078	15.55078
	Equal variances not assumed			1.697	2.185	.221	5.90000	3.47595	-7.90699	19.70699
Effect of M. oleifer extract on weight (g) of diabetic rats	Equal variances assumed	.315	.605	.953	4	.394	3.83333	4.02133	-7.33167	14.99834
	Equal variances not assumed			.953	3.645	.399	3.83333	4.02133	-7.77348	15.44014

T-Test (Week 2)

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of B. sapida extract on weight (g) of diabetic rats	CONTROL Diabetic	3	182.4667	1.95533	1.12891
	TREATED Diabetic	3	191.4333	.85049	.49103
Effect of M. oleifera extract on weight (g) of diabetic rats	CONTROL Diabetic	3	186.0000	.51962	.30000
	TREATED Diabetic	3	178.4333	1.15036	.66416

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of B. sapida extract on weight (g) of diabetic rats	Equal variances assumed	1.407	.301	-7.284	4	.002	-8.96667	1.23108	-12.38469	-5.54864
	Equal variances not assumed			-7.284	2.731	.007	-8.96667	1.23108	-13.11252	-4.82082
Effect of M. oleifera extract on weight (g) of diabetic rats	Equal variances assumed	.960	.383	10.383	4	.000	7.56667	.72877	5.54327	9.59007
	Equal variances not assumed			10.383	2.784	.003	7.56667	.72877	5.14192	9.99142

T-Test (Week 3)

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of B. sapida extract on weight (g) of diabetic rats	CONTROL Diabetic	3	185.3000	.91652	.52915
	TREATED Diabetic	3	198.6000	.91652	.52915
Effect of M. oleifera extract on weight (g) of diabetic rats	CONTROL Diabetic	3	179.7000	1.57162	.90738
	TREATED Diabetic	3	179.6333	6.57597	3.79664

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Effect of B. sapida extract on weight (g) of diabetic rats	Equal variances assumed	.000	1.000	-17.773	4	.000	-13.30000	.74833	-15.37770	-11.22230
	Equal variances not assumed			-17.773	4.000	.000	-13.30000	.74833	-15.37770	-11.22230
Effect of M. oleifera extract on weight (g) of diabetic rats	Equal variances assumed	8.170	.046	.017	4	.987	.06667	3.90356	-10.77135	10.90469
	Equal variances not assumed			.017	2.228	.988	.06667	3.90356	-15.18634	15.31968

T-Test (Week 4)

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Effect of B. sapida extract on weight (g) of diabetic rats	CONTROL Diabetic	3	182.1333	1.15036	.66416
	TREATED Diabetic	3	197.8333	1.59478	.92075
Effect of M. oleifera extract on weight (g) of diabetic rats	CONTROL Diabetic	3	175.9333	3.21455	1.85592
	TREATED Diabetic	3	177.6667	6.46091	3.73021

Independent Samples Test

		Levene's Test for quality of Variance		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	ig. (2-tailed	Mean Difference	Std. Error Difference	Lower	Upper
Effect of B. sa extract on wei (g) of diabetic	Equal varian assumed	.546	.501	-13.829	4	.000	5.70000	1.13529	8.85208	2.54792
	Equal varian not assumed			-13.829	3.638	.000	5.70000	1.13529	8.97977	2.42023
Effect of M. ol extract on wei (g) of diabetic	Equal varian assumed	3.014	.158	-.416	4	.699	-1.73333	4.16640	13.30111	9.83445
	Equal varian not assumed			-.416	2.933	.706	-1.73333	4.16640	15.16567	11.69901

General Linear Model

Warnings

Post hoc tests are not performed for Sample because there are fewer than three groups.

Between-Subjects Factors

		Value Label	N
Sample	1.00	CONTROL Diabetic	15
	2.00	TREATED Diabetic	15
Week	.00		6
	1.00		6
	2.00		6
	3.00		6
	4.00		6

Descriptive Statistics

	Sample	Week	Mean	Std. Deviation	N
Effect of B. sapida extract on weight (g) of diabetic rats	CONTROL Diabetic	.00	185.8667	1.46401	3
		1.00	193.4333	5.88586	3
		2.00	182.4667	1.95533	3
		3.00	185.3000	.91652	3
		4.00	182.1333	1.15036	3
		Total	185.8400	4.88990	15
	TREATED Diabetic	.00	186.9000	1.41067	3
		1.00	187.5333	1.26623	3
		2.00	191.4333	.85049	3
		3.00	198.6000	.91652	3
		4.00	197.8333	1.59478	3
		Total	192.4600	5.23611	15
	Total	.00	186.3833	1.40487	6
		1.00	190.4833	4.99416	6
		2.00	186.9500	5.09303	6
		3.00	191.9500	7.33069	6
		4.00	189.9833	8.68871	6
		Total	189.1500	6.00940	30
Effect of M. oleifera extract on weight (g) of diabetic rats	CONTROL Diabetic	.00	182.4667	1.95533	3
		1.00	185.6000	4.08534	3
		2.00	186.0000	.51962	3
		3.00	179.7000	1.57162	3
		4.00	175.9333	3.21455	3
		Total	181.9400	4.47960	15
	TREATED Diabetic	.00	181.6667	1.41892	3
		1.00	181.7667	5.64122	3
		2.00	178.4333	1.15036	3
		3.00	179.6333	6.57597	3
		4.00	177.6667	6.46091	3
		Total	179.8333	4.48548	15
	Total	.00	182.0667	1.58955	6
		1.00	183.6833	4.87992	6
		2.00	182.2167	4.22062	6
		3.00	179.6667	4.27629	6
		4.00	176.8000	4.66176	6
		Total	180.8867	4.53300	30

Multivariate Tests[c]

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	1.000	108047.6[a]	2.000	19.000	.000
	Wilks' Lambda	.000	108047.6[a]	2.000	19.000	.000
	Hotelling's Trace	11373.427	108047.6[a]	2.000	19.000	.000
	Roy's Largest Root	11373.427	108047.6[a]	2.000	19.000	.000
Sample	Pillai's Trace	.803	38.683[a]	2.000	19.000	.000
	Wilks' Lambda	.197	38.683[a]	2.000	19.000	.000
	Hotelling's Trace	4.072	38.683[a]	2.000	19.000	.000
	Roy's Largest Root	4.072	38.683[a]	2.000	19.000	.000
Week	Pillai's Trace	.935	4.388	8.000	40.000	.001
	Wilks' Lambda	.247	4.810[a]	8.000	38.000	.000
	Hotelling's Trace	2.314	5.207	8.000	36.000	.000
	Roy's Largest Root	1.934	9.669[b]	4.000	20.000	.000
Sample * Week	Pillai's Trace	1.022	5.223	8.000	40.000	.000
	Wilks' Lambda	.135	8.163[a]	8.000	38.000	.000
	Hotelling's Trace	5.229	11.766	8.000	36.000	.000
	Roy's Largest Root	4.997	24.984[b]	4.000	20.000	.000

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

c. Design: Intercept+Sample+Week+Sample * Week

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Effect of B. sapida extract on weight (g) of diabetic rats	946.328[a]	9	105.148	20.832	.000
	Effect of M. oleifera extract on weight (g) of diabetic rats	288.428[b]	9	32.048	2.085	.082
Intercept	Effect of B. sapida extract on weight (g) of diabetic rats	1073331.675	1	1073331.675	212653.2	.000
	Effect of M. oleifera extract on weight (g) of diabetic rats	981599.585	1	981599.585	63850.797	.000
Sample	Effect of B. sapida extract on weight (g) of diabetic rats	328.683	1	328.683	65.120	.000
	Effect of M. oleifera extract on weight (g) of diabetic rats	33.285	1	33.285	2.165	.157
Week	Effect of B. sapida extract on weight (g) of diabetic rats	136.840	4	34.210	6.778	.001
	Effect of M. oleifera extract on weight (g) of diabetic rats	175.031	4	43.758	2.846	.051
Sample * Week	Effect of B. sapida extract on weight (g) of diabetic rats	480.805	4	120.201	23.815	.000
	Effect of M. oleifera extract on weight (g) of diabetic rats	80.111	4	20.028	1.303	.303
Error	Effect of B. sapida extract on weight (g) of diabetic rats	100.947	20	5.047		
	Effect of M. oleifera extract on weight (g) of diabetic rats	307.467	20	15.373		
Total	Effect of B. sapida extract on weight (g) of diabetic rats	1074378.950	30			
	Effect of M. oleifera extract on weight (g) of diabetic rats	982195.480	30			
Corrected Total	Effect of B. sapida extract on weight (g) of diabetic rats	1047.275	29			
	Effect of M. oleifera extract on weight (g) of diabetic rats	595.895	29			

a. R Squared = .904 (Adjusted R Squared = .860)

b. R Squared = .484 (Adjusted R Squared = .252)

Estimated Marginal Means

Grand Mean

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Effect of B. sapida extract on weight (g) of diabetic rats	189.150	.410	188.294	190.006
Effect of M. oleifera extract on weight (g) of diabetic rats	180.887	.716	179.393	182.380

Post Hoc Tests

Week

Homogeneous Subsets

Effect of B. sapida extract on weight (g) of diabetic rats

	Week	N	Subset	
			1	2
Duncan [a,b,c]	.00	6	186.3833	
	2.00	6	186.9500	
	4.00	6		189.9833
	1.00	6		190.4833
	3.00	6		191.9500
	Sig.		.667	.166

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 5.047.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Effect of M. oleifera extract on weight (g) of diabetic rats

	Week	N	Subset	
			1	2
Duncan [a,b,c]	4.00	6	176.8000	
	3.00	6	179.6667	179.6667
	.00	6		182.0667
	2.00	6		182.2167
	1.00	6		183.6833
	Sig.		.220	.118

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 15.373.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Multiple Comparisons

Dependent Variable		(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Effect of B. sapida extract on weight (g) of diabetic rats	LSD	.00	1.00	-4.1000*	1.29709	.005	-6.8057	-1.3943
			2.00	-.5667	1.29709	.667	-3.2724	2.1390
			3.00	-5.5667*	1.29709	.000	-8.2724	-2.8610
			4.00	-3.6000*	1.29709	.012	-6.3057	-.8943
		1.00	.00	4.1000*	1.29709	.005	1.3943	6.8057
			2.00	3.5333*	1.29709	.013	.8276	6.2390
			3.00	-1.4667	1.29709	.272	-4.1724	1.2390
			4.00	.5000	1.29709	.704	-2.2057	3.2057
		2.00	.00	.5667	1.29709	.667	-2.1390	3.2724
			1.00	-3.5333*	1.29709	.013	-6.2390	-.8276
			3.00	-5.0000*	1.29709	.001	-7.7057	-2.2943
			4.00	-3.0333*	1.29709	.030	-5.7390	-.3276
		3.00	.00	5.5667*	1.29709	.000	2.8610	8.2724
			1.00	1.4667	1.29709	.272	-1.2390	4.1724
			2.00	5.0000*	1.29709	.001	2.2943	7.7057
			4.00	1.9667	1.29709	.145	-.7390	4.6724
		4.00	.00	3.6000*	1.29709	.012	.8943	6.3057
			1.00	-.5000	1.29709	.704	-3.2057	2.2057
			2.00	3.0333*	1.29709	.030	.3276	5.7390
			3.00	-1.9667	1.29709	.145	-4.6724	.7390
Effect of M. oleifera extract on weight (g) of diabetic rats	LSD	.00	1.00	-1.6167	2.26372	.483	-6.3387	3.1054
			2.00	-.1500	2.26372	.948	-4.8720	4.5720
			3.00	2.4000	2.26372	.302	-2.3220	7.1220
			4.00	5.2667*	2.26372	.031	.5446	9.9887
		1.00	.00	1.6167	2.26372	.483	-3.1054	6.3387
			2.00	1.4667	2.26372	.524	-3.2554	6.1887
			3.00	4.0167	2.26372	.091	-.7054	8.7387
			4.00	6.8833*	2.26372	.006	2.1613	11.6054
		2.00	.00	.1500	2.26372	.948	-4.5720	4.8720
			1.00	-1.4667	2.26372	.524	-6.1887	3.2554
			3.00	2.5500	2.26372	.273	-2.1720	7.2720
			4.00	5.4167*	2.26372	.027	.6946	10.1387
		3.00	.00	-2.4000	2.26372	.302	-7.1220	2.3220
			1.00	-4.0167	2.26372	.091	-8.7387	.7054
			2.00	-2.5500	2.26372	.273	-7.2720	2.1720
			4.00	2.8667	2.26372	.220	-1.8554	7.5887
		4.00	.00	-5.2667*	2.26372	.031	-9.9887	-.5446
			1.00	-6.8833*	2.26372	.006	-11.6054	-2.1613
			2.00	-5.4167*	2.26372	.027	-10.1387	-.6946
			3.00	-2.8667	2.26372	.220	-7.5887	1.8554

Based on observed means.

*. The mean difference is significant at the .05 level.

Appendix II: **FLOW CHART OF THE RESEARCH WORK**