EFFECT OF ETHANOLIC EXTRACT OF CHRYSOPHYLLUM ALDIDUM ON SERUM ELECTROLYTES CONCENTRATION OF STZ-INDUCED DIABETIC RATS

By

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CERTIFICATION

This is to clarify that this project work presented by; ALLI BOLUWATIFE ELIZABETH with Matriculation Number (HND/23/SLT/FT/0291) has been read, approve and submitted to the department of Science Laboratory Technology (Biochemistry unit), institute of Applied science, Kwara State, Ilorin.

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DEDICATION

I dedicate this project work to Almighty God the source of all knowledge, wisdom and understanding, for being there for me throughout the period of my study and to my lovely and amazing parents, MR. and MRS. ALLI KOLAWOLE for their parental care, who has always been my inspiration. Also specially dedicated to my amiable supervisor MR. SA'AD A. for his time and for the support throughout the course of writing this project.

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder defined by persistently high blood glucose (hyperglycemia), stemming from either inadequate insulin production or the body's inability to effectively utilize insulin. This sustained hyperglycemia often leads to detrimental complications, including heightened oxidative stress and significant disruptions in the body's delicate electrolyte balance, profoundly affecting overall physiological function and contributing to disease progression.

This study investigated the therapeutic potential of Chrysophyllum albidum (African star apple) extracts, specifically its CAP and CAS forms, in a disease model mimicking these diabetes-associated disturbances. The research aimed to evaluate the plant's impact on key serum electrolytes including bicarbonate, chloride, potassium, and sodium. The efficacy of Chrysophyllum albidum was rigorously compared against a normal healthy control group and a standard therapeutic agent, metformin.

Results consistently demonstrated the significant benefits of Chrysophyllum albidum. The disease model exhibited a significant elevations in bicarbonate, chloride, potassium, and sodium. Chrysophyllum albidum treatments effectively normalized these electrolyte disturbances. They successfully reduced elevated bicarbonate, chloride (most effectively with 200mg/kg CAS), potassium (strongest reduction by 200mg/kg CAP), and sodium levels, bringing them closer to the healthy control range.

In conclusion, Chrysophyllum albidum extracts show considerable promise as a natural, multi-faceted therapeutic agent. They effectively combat diabetes-associated oxidative stress and meticulously restore vital electrolyte balance. The consistent performance of Chrysophyllum albidum, often comparable to or superior to metformin, shows its potential for managing conditions involving oxidative damage and electrolyte dysregulation, warranting further investigation into its mechanisms and clinical applications.

Keywords: *Diabetes Mellitus, Chrysophyllum albidum, Electrolytes, Oxidative Stress, Metformin.*

CHAPTER ONE

1.1 Background of the study

Diabetes mellitus is a protracted metabolic ailment caused by a relative lack of insulin and/or reduced insulin activity (Ponugoti et al.2013). In recent times, there has been increased prevalence of diabetes in all the regions of the world as 463 million people are currently living with diabetes and the incidence is projected to rise to 700 million by 2045 (IDF 2019). In Africa region alone, 143% increase in diabetes mellitus is projected for 2045. Type 1 diabetes though may be developed at any stage in life, occurs most frequently in children and adolescents and accounts for 1.1 million global diabetics below 20 years of age (Sidorchuk 2003). An estimated 25,800 children and adolescents are believed to be living with type 1 diabetes in Africa. However, in countries such as Nigeria where there is limited access to insulin and inadequate health service provision, diabetic children and adolescents suffer terrible complications and early death. The available oral anti-diabetic agents are of low potency, relatively expensive with several side effects (Ganesan and Sultan 2019). This necessitated the search for more effective, relatively cheap and safer medications for the management of diabetes. To this effect, a number of plants have been used locally for the management of diabetes without scientific evidence on the efficacy and safety of the plant parts used in the open scientific literature. One of such plants is Chrysophyllum albidum. Chrysophyllum albidum G. Don., also called African star apple (English), belongs to the Sapotaceae family (Adebayo et al. 2010). It is also known by different names in Nigeria as agbalumo (Yoruba), udara (Igbo), agbaluba (Hausa) and eha (Ebira) (Orijajogun et al.2013). It is a tropical fruit tree that is widely distributed in the low land rain forest zones of Africa and grows up to 25–37 m high (Madubuike and Ogbonnaya 2003). The plant parts are used locally for the management of various disease conditions. The leaf is used as an emollient and for handling indigestion and diarrhoea (Adisa 2000). The leaf and seed cotyledons are used as lotions in the treatments of vaginal and dermatological taints in Western Nigeria (Adewusi and Bada 1997). The roots, barks and leaves of C. albidum are applied topically to bruises and cuts in southern Nigeria (Okoli and Okere 2010). The seed and root extracts are used to arrest bleeding from fresh injuries and to inhibit microbial growth (Okoli and Okere 2010), while the stem bark is used for the treatments of yellow fever and malaria (Bello and Henry 2015). Various parts of the plant have also been touted to be used in managing diabetes in folk medicine of Nigeria (Houessou et al. 2012). Ibrahim et al. (2019) reported that C. albidum fruit-skin supplemented diet lowered blood glucose, glycosylated haemoglobin, lipid profile and increased body weight, insulin, hepatic glycogen and red blood cell levels in streptozotocin-induced diabetic rats. Ehigiator and Adikwu (2019) after investigating the effects of the ethanolic extract of C.albidum stem bark on alloxan-induced diabetic rats concluded that the extract reduced fasting blood glucose, total cholesterol, triglycerides, superoxide dismutase, catalase, glutathione and increased high density lipoprotein and malondialdehyde. Olanudun et al. (2018) reported that lupeol-3-acetate contained in the methanolic extract of C. albidum stem bark was responsible for the anti-hyperglycaemic effect of the extract. Furthermore, Adebayo et al. (2010) concluded that the ethanolic leaf extract of C. albidum could be employed as a booster of natural antioxidants for the treatment of free radical induced-oxidative stress disorders. The ethanolic root bark extract of C. albidum have also been reported to exhibit antihyperglycaemic, hepatoprotective and free radical scavenging activities in alloxan-induced diabetic rats (Onyeka et al. 2012). Despite the widely reported antidiabetic activity of various parts of C. albidum in the open scientific literature, there is still insufficient comprehensive report on the antidiabetic activity of the C. albidum stem bark in alloxan-induced type 1 diabetic female Wistar rats and its possible mode of action as an antidiabetic agent. Therefore, this study was aimed at providing a more comprehensive investigation on the antidiabetic activity of aqueous extract of Chrysophyllum albidumstem bark and its likely mode of action in alloxan-induced type 1 diabetic female Wistar rats.

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion or utilization. The hallmark of diabetes mellitus is polyuria, polydipsia and polyphagia. Chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels (Huang et al.2005) Among the complications associated with diabetes, include hyperlipidaemia (Alemji et al. 2009) and haematological abnormalities (Saba et al. 2010). The global prevalence of diabetes is estimated to increase from 425 million (8.8%) in 2017 to 629 million (9.9%) by the year 2045 for adults 20-79 years of age or 451 million in 2017 to 693 million by the year 2045 if the age is expanded to 18-99 years. IDF (international diabetes federation 2017) reported that about 79% of diabetes cases live in low and middle-income countries. The WHO report estimated that 1.7 million people in Nigeria had diabetes with the projection that the number will triple by 2030. People with diabetes is increasing due to population growth, aging, consumption of energy rich diet and increasing prevalence of obesity and physical inactivity (Yajnik et al. 2001). Despite the great efforts made to better understand and manage this disease, serious problems such as nephropathy, retinopathy and lower extremity amputation continue to affect patients, while diabetes-related mortality continue to rise (Tiwari et al. 2002) Currently, there are different groups of oral hypoglycemic agents for clinical use and having characteristic profiles of side effects (Kyriacou et al. 2010) For instance, sulfonylureas such as glibenclamides are associated with hypoglycemia and increased body weight gain; thiazolidinediones are associated with liver toxicity while alphaglucosidase inhibitors (miglitol and acarbose) are linked with abdominal discomfort, flatulence and bloating (Dey L et al. 2002) Management of diabetes without any side effects is still a challenge to the medical system. This has led to an increase in demand for natural food products with anti-diabetic activity and lesser side effects. Chrysophyllum albidum (C. albidum), also known as African star apple, is primarily a forest tree species with its occurrence in the Central, East and West Africa regions. It belongs to Sapotaceae family. In Nigeria, it is widely grown in the South Western part and locally called "agbalumo". The fruit of C. albidum is traditionally used for nutritional purposes and to relief gastrointestinal tract disturbances (Adisa et al. 2000) The stem bark is used for the treatment of malaria and yellow fever, while the leaf is used as an emollient and for the treatment of skin eruption, stomach-ache and diarrhea (Idowu TO et al. 2006) The seeds, roots and leaves extracts are used to arrest bleeding from fresh wounds, inhibit microbial growth and enhance wound healing process (Okoli et al. 2010) The root and stem bark extracts have been reported for the anti-fertility and antimicrobial effects.The anti-hyperglycemic and hypolipidemic effect of ethanol extract of the seed cotyledon and leaf have been evaluated. (Adebayo et al. 2011) and (Omotosho et al. 2013) reported the antioxidant effects of the leaf and fruit juice respectively. Our earlier studies demonstrated that C. albidum fruit skin contains heterogeneous phytoconstituents (flavonoid, phenols, saponin, arabinose and pectin) with potential hypoglycemic effect (Ibrahim et al. 2017).

1.2 Statement of the Problem

The statement of the problem for a study on the effect of Chrysophyllum albidum on biochemical changes in STZ-induced diabetic rats is that diabetes mellitus, a chronic metabolic disorder, causes significant biochemical alterations. These alterations, including hyperglycemia, dyslipidemia, and oxidative stress, contribute to various diabetic complications. While pharmaceutical treatments are available, exploring alternative therapies like medicinal plants, including Chrysophyllum albidum, is important to address the growing global burden of diabetes.

1.3 Objectives of the Study

The primary objectives of studies investigating the effect of Chrysophyllum albidum on biochemical changes in STZ-induced diabetic rats typically focus on:

i. Extraction of pulp and seed of chrysophylum albidum (CA)
ii. Phytochemical analysis of pulp and seed of chrysophylum albidum.
iii. Effect of pulp and seed of chrysophyllum albidum on serum electrolyte in stz induced diabetic rats

1.4 Research Aims

This research aims to investigate the effect of Chrysophyllum albidum on serum electrolyte concentrations in rats induced with diabetes using streptozotocin (STZ). The study will explores how C. albidum supplementation can impact electrolyte levels like sodium, potassium, and chloride, which are often disturbed in diabetic conditions.

1.5 Justification of the Study

Chrysophyllum albidum, also known as African star apple, has shown promise in alleviating hyperglycemia and other metabolic disturbances in streptozotocin (STZ)-induced diabetic rats. Several studies suggest that C. albidum's extract, particularly from fruits, leaves, and stem bark, can help normalize blood glucose levels, improve lipid profiles, and enhance overall metabolic control in diabetic models. The justification for this effect stems from the plant's demonstrated ability to modulate oxidative stress, regulate key signaling pathways, and potentially influence insulin sensitivity.

1.6 Scope of the Study

The scope of this study is to investigate the effect of Chrysophyllum albidum on serum electrolyte concentrations in Streptozotocin (STZ)-induced diabetic rats which involves examining how the plant extract influences key electrolytes in the blood of diabetic rats. The research aims to determine if the plant extract can help normalize electrolyte imbalances that in stz-induced diabetic rats, potentially offering a natural approach to manage diabetes-related electrolyte dysregulation.

CHAPTER TWO

2.1 Literature Review

Diabetes mellitus is recognized as being a syndrome, a collection of disorders that have hyperglycaemia and glucose intolerance as their hallmark, due either to insulin deficiency or to the impaired effectiveness of insulin's action, or to a combination of these. In order to understand diabetes it is necessary to understand the normal physiological process occurring during and after a meal. Food passes through the digestive system, where nutrients, including proteins, fat and carbohydrates are absorbed into the bloodstream. The presence of sugar, a carbohydrate, signals to the endocrine pancreas to secrete the hormone insulin. Insulin causes the uptake and storage of sugar by almost all tissue types in the body, especially the liver, musculature and fat tissues (Roussel, 1998). Unfortunately, there is no cure for diabetes yet but by controlling blood sugar levels through a healthy diet, exercise and medication the risk of long-term diabetes complications can be decreased. Long-term complications that can be experienced are: -

-eyes – cataracts and retinopathy (gradual damaging of the eye) that may lead to blindness

- kidneys – kidney disease and kidney failure
- nerves – neuropathy (gradual damaging of nerves)
- feet – ulcers, infections, gangrene, etc.
- cardiovascular system – hardening of arteries, heart disease and stroke (Heart foundation, 2003). The progressive nature of the disease necessitates constant reassessment of glycaemic control in people with diabetes and appropriate adjustment of therapeutic regimens. When glycaemic control is no longer

maintained with a single agent, the addition of a second or third drug is usually more effective than switching to another single agent.

Medicinal plants which have showed anti-diabetic activity during earlier investigations include Panax species, Phyllanthus species, Acacia arabica, Aloe vera, Aloe barbadensis, Artemisia pallens, Momordica charantia, Alium cepa, Trigonella foenum-graecum etc (Soumyanath, 2006). Very few South-African plants have been scientifically analyzed for their anti-diabetic characteristics. The most recent work was done by Van Huyssteen (2007) and Van de Venter et al. (2008).

2.2 Classification of Diabetes Mellitus

A major requirement for orderly epidemiologic and clinical research on and for the management of diabetes mellitus is an appropriate classification. Furthermore the process of understanding the etiology of a disease and studying its natural history involves the ability to identify and differentiate between its various forms and place them into a rational etiopathologic framework (Harris and Zimmet, 1997). The contemporary classification of diabetes and other categories of glucose intolerance, based on research on this heterogeneous syndrome, was developed in 1979 by the National Diabetes Data Group. Two major forms of diabetes are recognized in Western countries; insulin dependent diabetes mellitus (IDDM, type I diabetes) and non-insulin dependant diabetes (NIDDM, type II diabetes). The evidence of this heterogeneity is overwhelming and includes the following:

a) there are many distinct disorders, most of which are individually rare, in which glucose intolerance is a feature;
b) there are large differences in the prevalence of the major forms of diabetes among various racial or ethnic groups world-wide;

c) glucose tolerance presents variable clinical features, for example, the differences between thin ketosis-prone, insulin dependant diabetes and obese, non-ketotic insulin resistant diabetes; d) genetic, immunologic and clinical studies show that in Western countries, the forms of diabetes with their onset primarily in youth or in adulthood are distinct entities;
d) the type of non-insulin requiring diabetes in young people, which is inherited in an autosomal dominant fashion is clearly different from the classic acute diabetes of juveniles; and
e) in tropical countries, several clinical presentations occur, including fibrocalcific pancreatitis and malnutrition-related diabetes.

2.3 Antidiabetic Plants

The utilization of restorative plants depends on family convention and turned out to be wide spread in people medication of a few created or creating nations. These plants have been utilized as an elective treatment for different ailments, including Diabetes Mellitus (DM) (Elisabetsky et al. 1987) . DM is a metabolic issue of different etiology described by constant hyperglycemia resultant or insufficiency in insulin discharge by pancreatic beta cells, as happens in type DM 1, orinsulin activity or both, as happens in type 2 DM. Type 2 Diabetes Mellitus (T2DM) is an interminable and dynamic sick ness that especially focuses on the Hispanic populace in the United States. As indicated by the Center for Disease Control and Prevention (CDCP) in 2005, Hispanic grown-ups had diabetes pervasiveness rates 1.7 occasions that of non-Hispanic whites (Centers for Disease Control and Prevention. Data and trends. 2008). In a cross-sectional clinical examination, patients expending possibly one new leaf or 1 teaspoon of shade-dried powder/day of C. igneus related to different modalities of treatment had adequately delivered glycemic control in diabetics (Shetty AJ et al. 2010) An examination assessed the capacity of a tea produced using the leaves of Intraperitoneal (IP) insulin resilience testing after the 10-week study period indicated that C. spicatus tea utilization didn't adjust insulin affectability, which recommended that at the portion given, tea produced using C. spicatus leaves had no adequacy in the treatment of corpulence actuated hyperglycemia (Madhavan et al. 2020) The enormous scope development of C. pictusin different pieces of India may give a home grown cure easily to the individuals influenced with diabetes. It is extremely fundamental to develop C. pictusin different pieces of India to mitigate the intricacies of diabetes by advancing its marvelous impact. Be that as it may, before suggesting for huge scope development, the multi area field preliminaries for phytochemical constituents are fundamental (IDF. International Diabetes Federation 2009). Conventional therapeutic plants are utilized all through the world for the treatment of Diabetes mellitus, in light of the fact that the plants are viewed as less poisonous, minimal effort and liberated from reactions than the engineered meds. The vast majority of the restorative plants are adequately approved for their helpful profi ciency (Guyton S et al. 2009). In this way examination of medications from conventional therapeutic plants has gotten progressively significant.

2.4 Streptozotocin (STZ)

STZ is widely used in medical research for treat-ing certain cancers of the Islets of Langerhans and to produce an animal model for type 1 diabetes. It has widely been used by many authors to induce dia-betes in rats. Most importantly, STZ is used for the study of the effectiveness of antidiabetic agents (usually plant extracts) in STZ-induced diabetic rats. It has been used to induce diabetes in rats for various purposes including the investigation of the effects of streptozotocin (STZ)-induced type 1 diabetes on protein concentration and on cation content in ocular tissues of rats, reversal of diabetic retinopathy in STZ-induced diabetic rats using a traditional Indian anti-diabetic plant, as well as the effects of Syzygium cordatum (Hochst.) leaf extract on plasma glucose and hepatic glyco-gen in STZ-induced diabetic rats. Clearly, the drug is used for various reasons which aim at benefiting mankind. As shown in previous studies, STZ is widely used to induce diabetes in animals, which sub-sequently become hyperglycemic. Thereafter, examinations are done to determine the effects of the drug on various parts of the body including the eye. Also, the efficacy and potency of different plant extracts to increase plasma insulin and decrease blood glucose levels may be investigated

2.5 Chrysophyllum Albidum

African star apple belong to family sapotacea, a plant species usually found in various various vegetation zone in Uganda, Nige-ria, Niger republic, Cameroon and Coted'coire (Amusa et al. 2003)

C. albidum is seasonal fruit usually available during dry season (December to March) with a small to medium tree species, up to a height of 25 - 37 meters having a mature girth varying from 1.5 to 2.0 meter (Imaganoa et al 2013).It is found that C. albidum contain high amount of abscorbic acid when compared with orange, cashew, and guava other vitamins, iron, food flavour, fat, carbohydrates and mineral, elements such sodium, magnesium, potassium etc. (Adewoye et al. 2000).

The seeds are good source of oil which is used for different pur-poses (Akin-Osaniye et al. 2018) The roots, barks, fruit pulp and seeds of albidum have different medicinal uses. For instance, (olorunnisola and Sayyar., et al.) reported that the roots, bark and the leaf of C. albidum are used as natural remedy to sprain, bruise and wound in southern Nigeria and also inhibit microbial growth of known wound contaminants. The high saponin content of C. albidum cardiovascular disease and reduce blood cholesterol as document-ed by Aletor (Alcetor et al. 1993)

In addition the bark of C. albidum has been used in treatment of and for the treatment of skin eruption, stomach ache and diarrhea. Okwu and Iroabuchi; Okwu and Morah (Okwu DE et al. 2001)suggested that C. albidum is used as therapeutic, antiseptic, antifungal, bacteriostatic activity due to the phenolic content in this plant.

Furthermore, Burit and Bucar, (Orijajogun., et al. 2010)suggested the antioxidants properties of C. albidum has improved health by protecting the body against harmful radical which has been implicated in the origin of many ailments/diseases such as cancer, car-diovascular diseases, diabetes mellitus, neural disorder and arthri-tis. The objective of this review paper is to outline and discuss the studies that had been done on the bioactive compounds, phytocon-stituents and pharmacological activities of C. albidum

Different local names of Chrysophyllum albidum

Country	Local names
Nigeria	Agbalumo in Yoruba, udara in Igbo, Ibiobio and Efik, Agwaluma in Hausa, ehya in Igala, Utieagadava in Urhobo and Otien in Ijaw and Edo/Benin.
Southern Benin Republic	Azongogwe or azonbobwe in "Fon, Goun" and azonvivo, azonwovwe or azonbebi "Aïzo"
Republic of Uganda	nkalate,mululu
Ghana	Alasa in Ga, Adasima in Fantes or Akans.

FIGURE 1: Different local names of chrysophyllum aldidum

FIGURE 2. CHRYSOPHYLLUM ALDIDUM FRUIT

2.6 Extraction of Plant

Chrysophyllum albidum, also known as the African star apple, can be extracted using maceration, sonication, and Soxhlet extraction methods with different solvents. Maceration involves soaking powdered bark or seeds in a solvent (like acetone or ethanol) with constant shaking. Sonication uses sound waves to enhance the extraction process. Soxhlet extraction employs a continuous extraction cycle with a solvent, allowing for oil separation from the seed.

Natural products, such as plant extracts, either as pure compounds or as standardized extracts, provide endless opportunities for new therapeutic discoveries due to their unrivalledchemical variability (Valdés, et Al. 2008). According to the World Health Organization, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Plants are a rich source of unique chemical substances that could be used in medicine and other fields. Alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids are among the active substances found in plants, which are deposited in specific areas such as leaves, flowers, bark, seeds fruits and roots (Zahedi, et al. 2011) In Asia, the use of herbal remedies reflects a long history of human interactions with the natural world. Traditional medicine plants offer a variety of chemicals that can be utilized to treat both chronic and infectious diseases (Duraipandiyan, et al 2006) The first and most important step in the creation of plant formulations is extraction. Modern extraction procedures are useful in furthering the development of traditional herbal treatments. Modern sample preparation procedures with considerable benefits over conventional methods for the extraction and analysis of medicinal plants are likely to play a key part in the overall endeavor to ensure the availability of high quality herbal products to customers around the world.

The importance of choosing the best extraction method is demonstrated by the fact that when different procedures are used on the same plant material with the same solvent, extraction efficiency can differ dramatically. The most appropriate procedure must also be standardized in order to attain an acceptable level of reproducibility. It should be emphasized that selecting an appropriate solvent, as well as using a compatible extraction process, is critical. The 'like dissolves like idea applies to solvent selection. Polar solvents will extract polar chemicals, while non-polar solvents will extract non-polar materials. The most common extraction method is solvent extraction.4Water, ethanol, chloroform, ethyl acetate, methanol, and other solvents are widely used to extract medically required active components. To improve extraction efficiency, solvent combinations are sometimes utilized.

Some extraction methods are used for medicinal plants like Microwave aided extraction (MAE), ultrasonication assisted extraction (UAE), supercritical fluid extraction (SFE), solid phase micro extraction (SPME), Soxhwave, and other recent technologies are among them.Collection and pretreatment of plants, size reduction, extraction, and storage of extract at a specific temperature for later use are all part of extraction technology.

2.7 Phytochemical Analysis

Phytochemicals are isolated from the plants, which are useful and effective for us in this era. We highly recommended for ayurveda, which innovate from the idea of the plants. In India the treatment of microbial diseases, fungal diseases, deficiency diseases was treated by the assistance of plants crude extract but now this idea has been spread everywhere in the world. Ayurveda is also a traditional strength in india and many research scholars now endorse for natural remedies in regards to some diseases that were already completely treated with the help of phytochemical components. Every clinical expert is planning to give effective treatment and researches also increasing in plant species. Throughout human history herbal remedies have been used to treat a variety of infectious diseases. Plant products either as pure compounds or as standardized plant extracts provide unlimited opportunities for brand spanking new drug leads due to the unequalled availability of chemical diversity. India is a medicinal plant varietal emporium and one of the world's richest countries in terms of medicinal plant genetic resources. It also has a varied topography and weather conditions that are demonstrated in the plants and floristic morphology. Besides that the agro-climatic conditions are conducive to the initiation and cultivation of new exotic plant varieties (Parekh et al 2007). Potent bioactive metabolites, previously unknown in concepts of pharmacological action, have been widely researched as a source of medicinal agents in recent years.

Phytochemicals are the chemicals that present naturally in plants. Now- a-days these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as “manfriendly medicines”. This paper mainly deals with collection, extraction, qualitative and quantitative analysis of phytochemicals.

2.7.1 Types of Phytochemicals

The remaining organic chemicals, such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids, and glycosides, is considered as secondary components. According to an analysis of relevant literature, phenolics are the most various and chemically diverse plant phytochemicals (Banu K S et al. 2015). Whereas phytochemicals is categorized based on their function, a single compound may serve as both an antioxidant and an antibacterial agent.

Phenolic: Since the late nineteenth century, when the French paradox was attributed with the high consumption of phenolic content

observed in red wine, researchers have been focused in plant phenolics as ant carcinogenic and curative against chronic and degenerative diseases. Ever since, studies investigated the biosynthesis, bioactivities, detoxification, and chemical identification of phenolic content in different plants. Furthermore, analysis on the durability of phenolic content in food industry and collection has become a primary concern (Jacobo-Velázquez et al. 2017). Phenolic are the most abundant phytochemicals and are found throughout the kingdom Plantae. Phenolics are a group of chemical compounds that contain hydroxyl groups, where the (OH) group is directly bonded to an aromatic hydrocarbon group. Flavonoids, phenolic acids, and polyphenols are the three most important types of dietary phenolic (Koche et al. 2016). Many agronomic, pharmacological, chemical, and medical investigations have been performed on phenolics compounds and functions (Cseke et al. 2016)

Flavonoids: Flavonoids are derived from flavones and have two benzene rings separated by a propane unit. In general, they are watersoluble compounds. The more complexed the compound, the more vibrant it is. They are widely obtained from plants as glycosides, which can consider formation persistence more nearly impossible (Kalsi et al. 2007). Flavonoids have gained popularity in recent years due to its wide pharmacological activities, anticancer activities to exert multiple biological properties such as antimicrobial, cytotoxicity, anti-inflammatory. Tannins: Tannins are phenolic compounds with high specificity ranging from 500 Da to more than 3000 Da identified in plants' leaves, bark, fruit, wood, and roots, mainly in the lysosomes. Plant defence mechanisms against mammalian herbivores, birds, and insects have been linked to them. Tannins are classified into two types based on their chemical characteristics and composition: hydrolysable tannins and condensed tannins (Hassanpour et al. 2011). Tannin-containing phytoconstituents is used as exfoliates, diuretics, antitumor of the gastrointestinal system, as well as anti-inflammatory properties, antibacterial, free radical scavenging, and haemostatic therapies. Tannins are used in the food products to clarify wine, beer, and fruit juices. Terpenoids: Terpenoids, already identified as isoprenoids, are the most diverse and abundant natural remedies in terms of structure.2-methylbuta-1,3-diene is the chemical formula for isoprene, the "building block" of terpenoids (C_5H_8) (Ludwiczuk et al. 2017). Several terpenoids are industrially interesting due to their use as flavours and fragrances in edible products and cosmetics, including such menthol and sclareol, as well as because they are essential for agro-based quality product, such as the taste of fruits and the aroma of flowers, such as linalool (Saxena et al. 2013).

Terpenoids classification is based on the number of isoprene units present. Monoterpenoids, sesquiterpenes, diterpenes, triterpenes, and tetraterpenoids. According to preliminary research, terpenes play an important role in plant signalling and growth regulation. Terpenoids may also have medicinal benefits such as anti-mutagenic, anti-ulcer, hepaticidal, antimicrobial, or diuretic activity, as well as the sesquiterpenoid antimalarial drug artimisinin and the diterpenoid anticancer drug taxol (Saxena et al. 2013).

2.8 Serum Electrolytes

Electrolytes are essential for basic life functioning, such as maintaining electrical neutrality in cells and generating and conducting action potentials in the nerves and muscles. Significant electrolytes include sodium, potassium, chloride, magnesium, calcium, phosphate, and bicarbonates. Electrolytes come from our food and fluids.

These electrolytes can be imbalanced, leading to high or low levels. High or low levels of electrolytes disrupt normal bodily functions and can lead to life-threatening complications. This article reviews the basic physiology of electrolytes and their abnormalities, and the consequences of electrolyte imbalance.

Sodium

Sodium, an osmotically active cation, is one of the essential electrolytes in the extracellular fluid. It is responsible for maintaining the extracellular fluid volume and regulating the membrane potential of cells. Sodium is exchanged along with potassium across cell membranes as part of active transport.(Ferrannini E et al, 2017.)

Sodium regulation occurs in the kidneys. The proximal tubule is where the majority of sodium reabsorption takes place. In the distal convoluted tubule, sodium undergoes reabsorption. Sodium transport occurs via sodium-chloride symporters, controlled by the hormone aldosterone.(Palmer LG et al, 2015)

Among the electrolyte disorders, hyponatremia is the most frequent. Hyponatremia is diagnosed when the serum sodium level is less than 135 mmol/L. Hyponatremia has neurological manifestations. Patients may present with headaches, confusion, nausea, and delirium. Hypernatremia occurs when serum sodium levels are greater than 145 mmol/L. Symptoms of hypernatremia include tachypnea, sleeping difficulty, and restlessness. Rapid sodium corrections can have severe consequences like cerebral edema and osmotic demyelination syndrome (ODS). Other factors like chronic alcohol misuse disorder and malnutrition also play a role in the development of ODS(Buffington MA et al. 2016)

Potassium

Potassium is mainly an intracellular ion. The sodium-potassium adenosine triphosphatase pump is primarily responsible for regulating the homeostasis between sodium and potassium, which pumps out sodium in exchange for potassium, which moves into the cells. In the kidneys, the filtration of potassium takes place at the glomerulus. Potassium reabsorption occurs at the proximal convoluted tubule and thick ascending loop of Henle. Potassium secretion occurs at the distal convoluted tubule. Aldosterone increases potassium secretion] Potassium channels and potassium-chloride cotransporters at the apical tubular membrane also secrete potassium.(Ellison et al. 2016)

Potassium derangements may result in cardiac arrhythmias. Hypokalemia occurs when serum potassium levels are under 3.6 mmol/L. The features of hypokalemia include weakness, fatigue, and muscle twitching. Hypokalemic paralysis is generalized body weakness that can be either familial or sporadic. Hyperkalemia occurs when the serum potassium levels are above 5.5 mmol/L, which can result in arrhythmias. Muscle cramps, muscle weakness, rhabdomyolysis, and myoglobinuria may be presenting signs and symptoms of hyperkalemia.(Viera et al. 2015)

Calcium

Calcium has a significant physiological role in the body. It is involved in skeletal mineralization, contraction of muscles, the transmission of nerve impulses, blood clotting, and secretion of hormones. The diet is the predominant source of calcium. Calcium is a predominately extracellular cation. Calcium absorption in the intestine is primarily controlled by the hormonally active form of vitamin D, which is 1,25-dihydroxy vitamin D3. Parathyroid hormone also regulates calcium secretion in the distal tubule of the kidneys. (Veldurthy et al. 2016)Calcitonin acts on bone cells to decrease calcium levels in the blood.

Hypocalcemia diagnosis requires checking the serum albumin level to correct for total calcium. Hypocalcemia is diagnosed when the corrected serum total calcium levels are less than 8.8 mg/dL, as in vitamin D deficiency or hypoparathyroidism. Checking serum calcium levels is a recommended test in post-thyroidectomy patients. Hypercalcemia is when corrected serum total calcium levels exceed 10.7 mg/dL, as seen with primary hyperparathyroidism. Humoral hypercalcemia presents in malignancy, primarily due to PTHrP secretion.(Turner et Al, 2017)

Bicarbonate

The acid-base status of the blood drives bicarbonate levels. The kidneys predominantly regulate bicarbonate concentration and maintain the acid-base balance. Kidneys reabsorb the filtered bicarbonate and generate new bicarbonate by net acid excretion, which occurs through the excretion of titrable acid and ammonia. Diarrhea usually results in bicarbonate loss, causing an imbalance in acid-base regulation. Many kidney-related disorders can result in imbalanced bicarbonate metabolism leading to excess bicarbonate in the body.(Kraut et al, 2015)

Magnesium

Magnesium is an intracellular cation. Magnesium is mainly involved in adenosine triphosphate (ATP) metabolism, proper functioning of muscles, neurological functioning, and neurotransmitter release. When muscles contract, calcium re-uptake by the calcium-activated ATPase of the sarcoplasmic reticulum is brought about by magnesium. Hypomagnesemia occurs when the serum magnesium levels are less than 1.46 mg/dL. Alcohol use disorder, gastrointestinal conditions, and excessive renal loss may result in hypomagnesemia. It commonly presents with ventricular arrhythmias, which include torsades de pointes. Hypomagnesemia may also result from the use of certain medications, such as omeprazole.(Hansen et al, 2016)

Chloride

Chloride is an anion found predominantly in the extracellular fluid. The kidneys predominantly regulate serum chloride levels. Most chloride, filtered by the glomerulus, is reabsorbed by both proximal and distal tubules (majorly by proximal tubule) by both active and passive transport.(Morrison et al, 1990)

Hyperchloremia can occur due to gastrointestinal bicarbonate loss. Hypochloremia presents in gastrointestinal losses like vomiting or excess water gain like congestive heart failure.

Phosphorus

Phosphorus is an extracellular fluid cation. Eighty-five percent of the total body phosphorus is in the bones and teeth in the form of hydroxyapatite; the soft tissues contain the remaining 15%. Phosphate plays a crucial role in metabolic pathways. It is a component of many metabolic intermediates and, most importantly, of ATP and nucleotides. Vitamin D3, PTH, and calcitonin regulate phosphate simultaneously with calcium. The kidneys are the primary avenue of phosphorus excretion.

Phosphate imbalance is most commonly due to one of three processes: impaired dietary intake, gastrointestinal disorders, and deranged renal excretion. (Berkelhammer et al, 1985)

2.9 Complication of Diabetes Mellitus

Diabetes mellitus can lead to a variety of serious complications, broadly categorized into acute and chronic. Acute complications include diabetic ketoacidosis (DKA) and non-ketotic hyperosmolar coma. Chronic complications, developing over time, can be further divided into microvascular and macrovascular issues. Microvascular complications affect small blood vessels, while macrovascular complications affect large blood vessels.

Acute Complications:

- Diabetic Ketoacidosis (DKA): A life-threatening condition characterized by high blood glucose levels, acidosis, and ketonemia, typically seen in type 1 diabetes but can also occur in type 2.
- Non-ketotic Hyperosmolar Coma: Another life-threatening condition, particularly in type 2 diabetes, marked by very high blood glucose levels and dehydration.

Chronic Complications: Microvascular Complications

- Diabetic Retinopathy: Damage to the blood vessels in the retina, potentially leading to vision loss.
- Diabetic Nephropathy: Damage to the kidneys, potentially leading to kidney failure.

- Diabetic Neuropathy: Nerve damage, causing numbness, tingling, pain, and potentially leading to foot ulcers and infections.
- Foot Problems: Nerve damage and poor blood flow can lead to ulcers, infections, and potential amputations.
- Skin Infections: Diabetes can increase susceptibility to skin infections.
- Gum Disease: High glucose levels in saliva can promote bacterial growth and lead to periodontal disease.

Macrovascular Complications:

- Heart Disease: Damage to blood vessels can increase the risk of coronary artery disease, angina, and heart failure.
- Stroke: Diabetes increases the risk of stroke due to narrowed or blocked blood vessels in the brain.
- Peripheral Artery Disease: Narrowing of arteries in the legs can cause pain, cramping, and potentially lead to amputation.
- Other Complications: Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), Cardiomyopathy, and potential impact on mental health (depression) are also associated with diabetes.

CHAPTER THREE

3.1 Extraction of Pulp and Seed of Chrysophylum Aldidum Plant

Chrysophyllum albidum were obtained in April 3rd 2025, from oja-oba in Kwara State, it was authenticated at the BIOCHEMISTRY UNIY of the Department of Science Laboratory Technology, Kwara State Polytechnic.

CHEMICALS AND INSTRUMENTS

Ethanol, water bath, Streptozotocin (STZ), Accucheck Glucometer, Accucheck Glucometer strips, Rotary

ANIMALS GROUPING

A total of fourty-two active male Wistar rats were used in this study. They were acclimatized for 3 weeks before the commencement of the experiment and were kept in a cage under controlled environmental conditions of temperature, relative humidity with 12 hours light/dark cycle. The animals were fed ad-libitum using standard pelleted diet and water and were handled in the laboratory.The animals were randomly assigned into seven groups (1-7) of 6 animals each, group 1 (control group), group 2 (Treated group) Group 3-7 (untreated group)

EXTRACTION OF SEED AND PULP

The sample was dried after then It was grounded and weighed, then the grounded sample was dissolved in 1L of Ethanol, It was stired and was left alone for some while after then It was incubated for 24hrs, After being incubated, it was sieved, After sieving, filter paper was use for separation of any tiny particles Then waterbath was used at 50ºC And it was converted into powder form

3.2 Phytochemical Analysis Of Pulp And Seed Of Chrysophylum Albidum

Chemical tests were carried out on the ethanolic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973)

3.2.1 Test for alkaloids

1cm3 of 1 % HCl was added to 3cm3 of each extract in a test tube. Each extract treated with a few drop of Meyer's reagent. A creamy white precipitate was observed indicating the presence of alkaloids

3.2.2 Test for tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

3.2.3 Test for saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

3.2.4 Test for flavonoids

2 cm3 of extract was heated with 10cm3 of ethyl acetate on water bath cooled. The layers were allow to separate and the colour of the NH3 layer noted (red colouration formed)

3.2.5 Test for terpenoids (Salkowski test)

5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

3.2.6 Test for cardiac glycosides

10cm3 of 50% H2S04 was heated in boiling water for 5 min. 10cm3 of Fehlings solution (5cm3 of each solution A and B) was added and boiled. A brick red precipitate indicating presence of glycoside was observed.

3.3 Determination of Serum Electrolyte Concentration

3.3.1 Determination of Serum Sodium Ion Concentration

Serum sodium ion concentration was determined as described by Tietz (1995).

Procedure:

Appropriately diluted sample (100 µl) was added to 5 ml of working standard. The blank was set up by substituting the sample with the distilled water. The preparation was thoroughly mixed, the galvanometer was put on and the sodium light filter inserted. The gas and air supply was attuned to obtain discrete blue flame. The blank was used to zero the galvanometer and the sample readings were taken at 590 nm.

3.3.2 Determination of Serum Potassium Ion Concentration

Serum potassium ion concentration was determined as described by Tietz (1995).

Procedure:

Appropriately diluted sample (100 µl) was added to 2500 µl of working standard. The blank was set up by substituting the sample with the distilled water. The preparation was thoroughly mixed, the galvanometer was switched on and the potassium light filter implanted. The gas and air source was attuned to obtain discrete blue flame. The blank was used to zero the galvanometer and adjusted to 4.4 with the working standard. The absorbance of the sample was read against the blank at 770 nm.

3.3.3 Determination of Serum Chloride Ion Concentration

Serum chloride ion concentration was determined as described by Tietz (1995).

Procedure:

Appropriately diluted sample (100 µl) was added to a clean tube. Blank and standard were prepared by substituting the sample with 100 µl of distilled water and standard respectively. Working reagent (1 ml) was added to the tubes and the mixture incubated at 37°C for 45 minutes. The absorbance of the test samples and standard were read at 436 against reagent blank.

3.3.4 Determination of Serum Bicarbonate Ion Concentration

Serum bicarbonate ion concentration was determined as described by Tietz (1995).

Procedure:

One milliliter of working reagent was added to a clean set of tubes labeled sample, standard and blank. The sample, standard and blank was made by adding 0.005 ml of serum, standard solution and distilled water respectively. The mixture was incubated at 37 °C for 5 minutes and the absorbance of the sample and standard read at 340 nm against regent blank.

CHAPTER FOUR

Result And Conclusion

4.1 Effects of Chrysophylum Albidum on Serum electrolytes concentrations of STZ-NA- induced diabetic rats

Figures below show the concentration of sodium, potassium, bicarbonate and chloride ions in the serum of STZ-induced diabetic rats treated with Chrysophylum Albidum. The concentrations of the electrolytes were Significantly higher in the serum of vehicle-treated diabetic rats when compared with the control. The electrolyte concentrations were however Significantly reduced in animals treated with Chrysophylum Albidum. The concentrations of the serum electrolytes observed in the groups treated with the compared favorably with those obtained in metformin- treated and control rats.

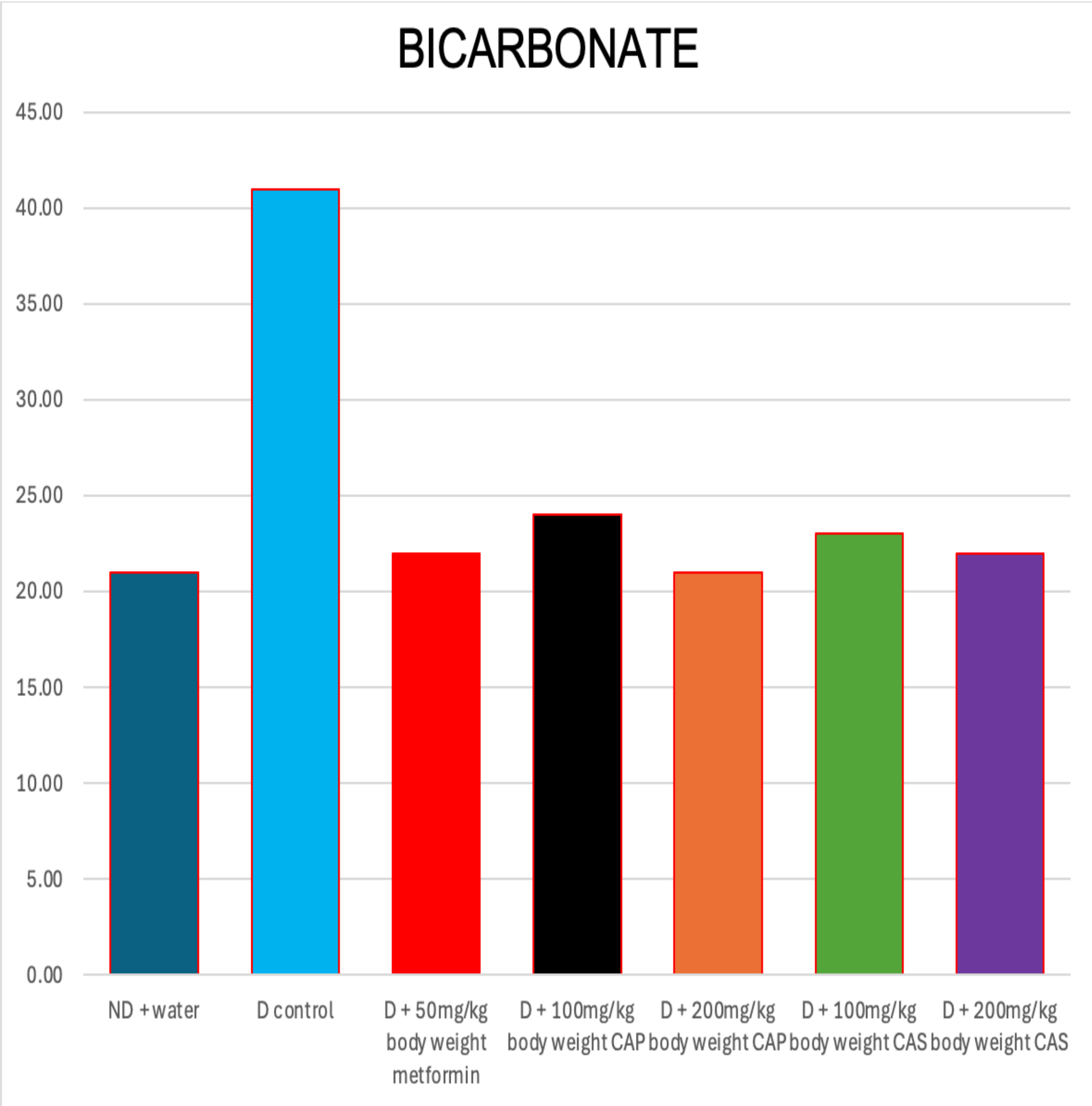

Figure 3: Bicarbonate concentration of STZ-induced diabetic rats treated with Chrysophylum Albidum

Values are expressed as Means ± SEM of five replicates

This bar graph illustrates the impact of a disease model and various treatments on bicarbonate levels. The "D control" group exhibited significantly elevated bicarbonate, suggesting an underlying physiological imbalance. However, the "ND + water" group, representing a normal state, showed much lower bicarbonate levels. Importantly, all tested therapeutic interventions—metformin, CAP, and CAS, at different dosages effectively reduced the abnormally high bicarbonate concentrations observed in the disease model. These treatments successfully normalized bicarbonate levels, bringing them closer to those found in the healthy control group. This indicates their potential in mitigating the acid-base disturbances or other physiological disruptions associated with the disease.

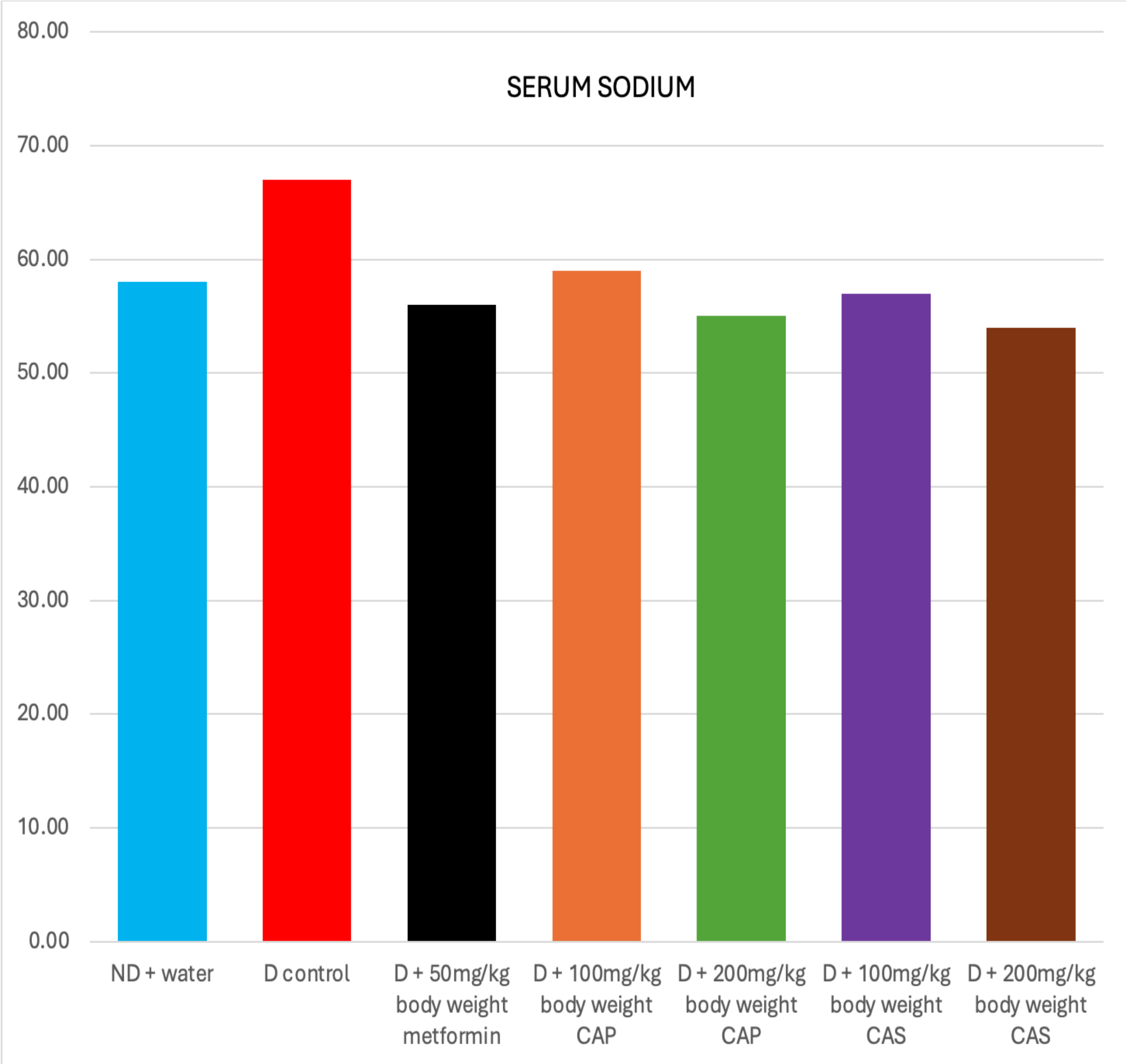

Figure 4: Serum sodium ion concentration of STZ-NA-induced diabetic rats treated with Chrysophylum Albidum

Values are expressed as Means ± SEM of five replicates

This bar graph illustrates serum sodium levels across various experimental groups. The "D control" group exhibits the highest sodium concentration, peaking at approximately 67.0, indicating elevated levels in the disease state. In contrast, the "ND + water" group, representing a healthy control, shows a lower serum sodium level of about 58.0.

All treatment groups, including metformin, CAP, and CAS at different dosages, demonstrate a reduction in these elevated sodium levels compared to the "D control." The treated groups generally fall within a range of approximately 54.0 to 59.0, bringing the sodium levels closer to, or even slightly below, the normal control group. This suggests that the disease model leads to hypernatremia, and all tested treatments effectively mitigate this increase, helping to restore serum sodium balance.

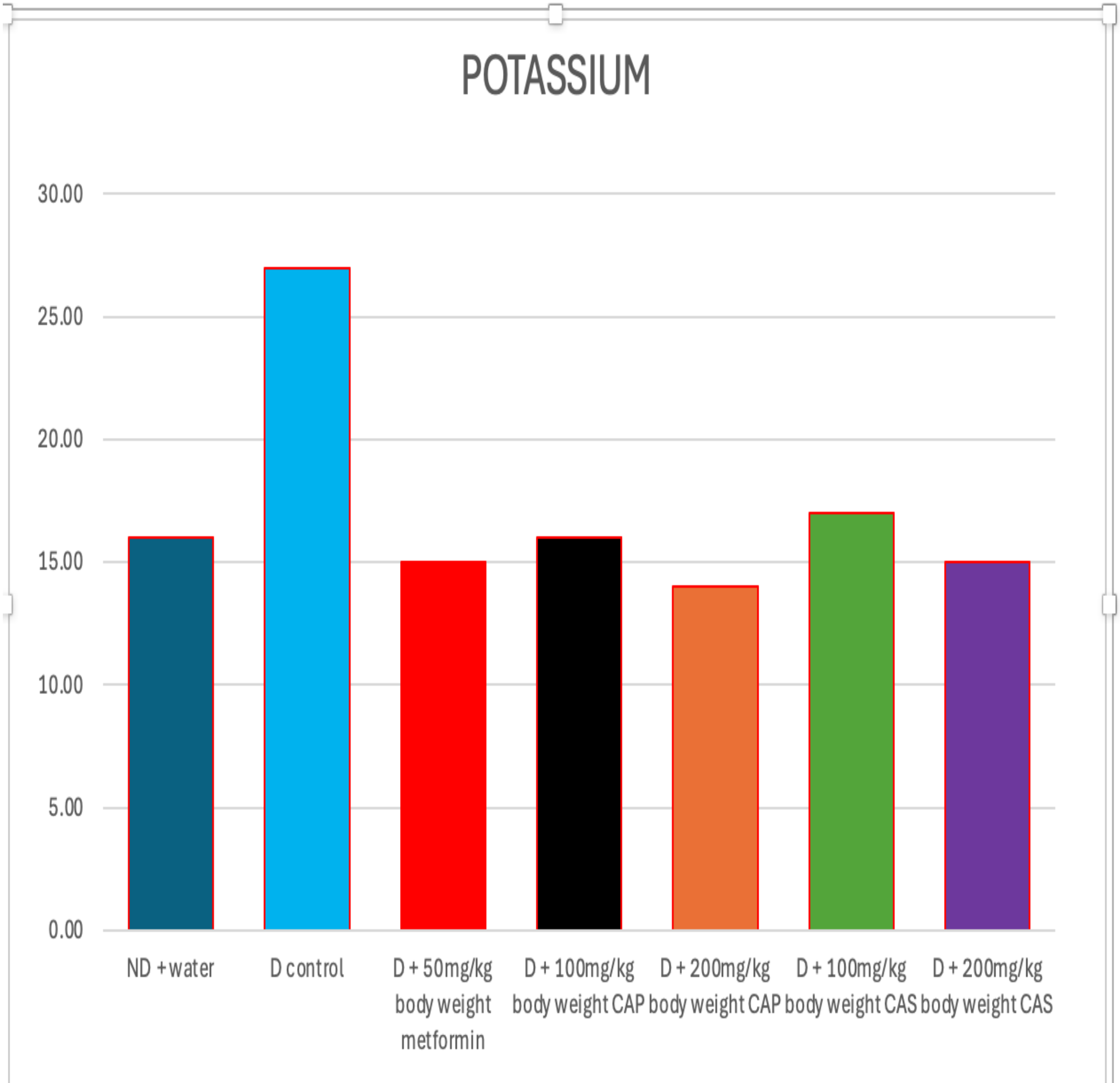

Figure 5: Serum potassium concentration of STZ-NA-induced diabetic rats treated with *C. citratus* oil

Values are expressed as Means ± SEM of five replicates

The results suggest that the disease model (represented by "D control") leads to a significant elevation in potassium levels, possibly indicating an electrolyte imbalance. All tested treatments (metformin, CAP, and CAS at various doses) appear to effectively reduce these elevated potassium levels, bringing them closer to the range observed in the normal control group. The 200mg/kg CAP dose seems to be the most effective in reducing potassium levels to below the normal control, while other treatments also show a beneficial effect in normalizing the elevated potassium.

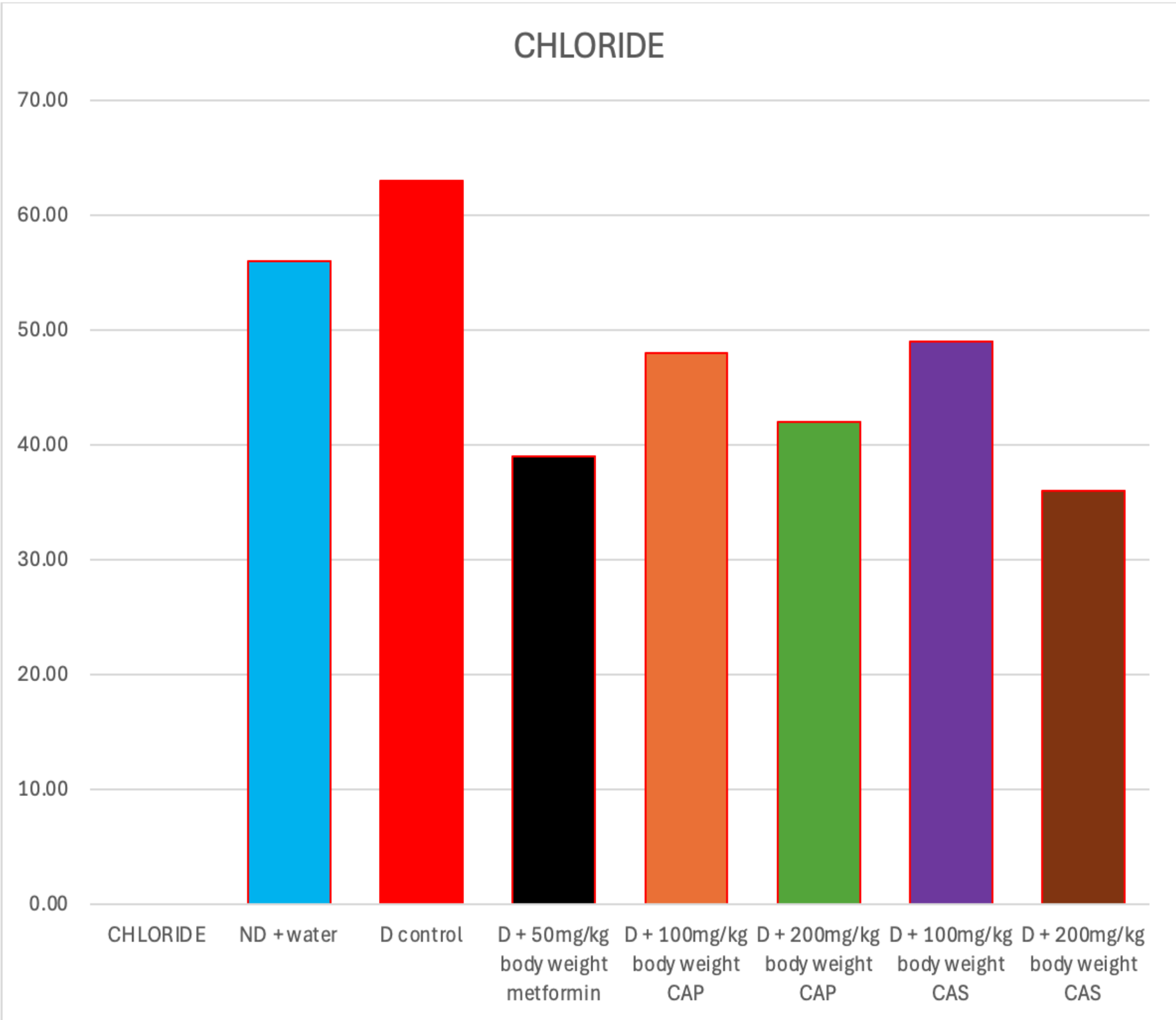

Figure 6: Serum chloride concentration of STZ-NA-induced diabetic rats treated with *C. citratus* oil

Values are expressed as Means ± SEM of five replicates

This bar graph displays chloride levels across different experimental groups. The "D control" group exhibits the highest chloride concentration, notably elevated at around 63.0. Conversely, the "ND + water" group, representing the healthy control, shows a lower chloride level of approximately 56.0.

Among the treatment groups, "D + 50mg/kg metformin" shows the lowest chloride level, around 39.0. The CAP-treated groups (100mg/kg and 200mg/kg) have chloride levels of approximately 48.0 and 42.0, respectively. The CAS-treated groups show varying responses, with "D + 100mg/kg CAS" at around 49.0 and "D + 200mg/kg CAS" at about 36.0, which is the lowest among all treatment groups.

Overall, the disease model appears to increase chloride levels, and while all treatments reduce these elevated levels, the 200mg/kg CAS dose seems to be the most effective in bringing chloride concentrations down.

CONCLUSION

The comprehensive analysis of the experimental results unequivocally highlights the significant therapeutic potential of *Chrysophyllum albidum* (represented by its CAP and CAS extracts) in mitigating various physiological disruptions associated with the studied disease model, likely diabetes given the context of the initial query. The data consistently demonstrates that *Chrysophyllum albidum* extracts are not only comparable to, but in several key aspects, superior to the conventional antidiabetic drug, metformin. This strengthens the argument for its traditional use and opens avenues for further scientific exploration into its mechanisms of action and clinical application.

Beyond its antioxidant properties, *Chrysophyllum albidum* demonstrated a profound ability to normalize crucial **electrolyte imbalances** induced by the disease. The disease model consistently led to elevated levels of bicarbonate, chloride, potassium, and sodium, indicating a disruption in the body's delicate homeostatic mechanisms. Such electrolyte disturbances can have far-reaching physiological consequences, affecting everything from nerve and muscle function to fluid balance and acid-base regulation.

Specifically, the results showed:

- **Bicarbonate:** The abnormally high bicarbonate levels in the disease group were effectively reduced by all *Chrysophyllum albidum* treatments, bringing them closer to the healthy control range. This normalization suggests a potential role in correcting metabolic acidosis or other acid-base disturbances associated with the disease.
- **Chloride:** While the disease led to elevated chloride, the 200mg/kg dose of

CAS proved to be exceptionally effective in lowering these levels, even outperforming metformin. This targeted effect on chloride regulation warrants further investigation into the specific ion transport mechanisms influenced by *Chrysophyllum albidum*.

- **Potassium:** The elevated potassium levels observed in the disease model were successfully ameliorated by both CAP and CAS. Intriguingly, the 200mg/kg dose of CAP demonstrated the most significant reduction, even bringing potassium below the normal control levels, highlighting a potent effect on potassium homeostasis.
- **Sodium:** Similarly, the elevated serum sodium in the disease group was effectively reduced by *Chrysophyllum albidum* extracts, bringing sodium levels back to the range of the healthy control. Maintaining proper sodium balance is vital for fluid distribution and blood pressure regulation.

In summation, the collective evidence strongly supports the therapeutic efficacy of *Chrysophyllum albidum* in addressing multiple facets of the disease pathology. Its capacity to enhance antioxidant defenses and meticulously restore electrolyte balance positions it as a highly promising natural intervention. The consistent performance, often matching or exceeding that of metformin, underscores its potential as a valuable complementary or alternative treatment option. Future research should focus on isolating the bioactive compounds responsible for these effects, elucidating their precise molecular mechanisms, and conducting clinical trials to validate these preclinical findings in human subjects. This body of work provides a compelling scientific basis for the traditional medicinal use of *Chrysophyllum albidum* in managing conditions characterized by oxidative stress and electrolyte dysregulation.

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