**TITLE PAGE**

**EFFECT OF *MOMORDICA CHARANTIA* LEAF FRACTIONS ON SERUM ELECTROLYTES AND RENAL FUNCTION INDICES IN ALLOXAN-INDUCED DIABETIC ALBINO RATS.**

**BY**

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**BIOCHEMISTRY**

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**JULY 2018.**

**APPROVAL**

This is to certify that this work has been supervised and approved for the project work in partial fulfilment of the requirement for the award of Bachelor of Science (B.Sc.) Biochemistry

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 ...........................

**STUDENT DATE**

NWANKWO, CHINELO QUEENDALINE

 **DEDICATION**

My project work is dedicated to God, the One above, who blesses us abundantly every day. May this project, and those who stood by me till the completion of this work be inspired to Give God Gratitude daily.

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**ABSTRACT**

Diabetes mellitus is the most common metabolic disorder in the community, plant like *Momordica charantia* can be used to correct this disorder. *Momordica charantia*, also known as bitter melon is a member of the family *Cucurbittaceae*. Seed, fruit and leave of this plant contain bioactive compounds with a wide range of biological activities that have been used in traditional medicine, in the treatment of several diseases including inflammation, infection and diabetes. The aim of this study is to investigate the effect of *Momordica charantia* fractions on some serum electrolytes and renal function indices in an alloxan induced albino rats. Fourty albino rats (180-274 g) were randomly divided into 8 groups with five in each group. The rats were injected intra-peritoneally with single dose of 100 mg/kg alloxan induced. Multiple doses 200 mg/kg of various fractions of plant extract were administered orally once daily for 14days. The rats were then sacrificed at 14th day and blood was collected for renal function test and electrolytes analysis using spectrophotometric method. The result showed that there was significant decrease (p>0.05) in srum level ( Na, K, Cl-) and in renal function indices ( Urea and Creatinine) as compared to the diabetic untreated groups. In conclusion, it has been shown that the leaf fractions of *momordica charantia* has a significant difference (P>0.05) in serum electrolytes and renal function indices.

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**CHAPTER ONE**

**INTRODUCTION**

**1.1 Background of study**

Diabetes affects one hundred and thirty five million people in one year worldwide (King et.al., 1999) and this figure is projected to rise to three hundred million in 2025 ((King et. al.,1998). It is obvious that diabetes, a chronic non communicable disease, continues to have a tremendous impact on society in terms of the quality of life and straining health care resources. The costs incurred in managing or preventing it are enormous, both in Kenya and throughout the world. The disease causes substantial morbidity, mortality and long-term complications and remains a risk factor for cardiovascular disease. In Africa, this disease continues to impact on the poverty levels of the people.

Diabetes mellitus is a systemic metabolic disorder characterized by elevated blood glucose due to absolute oxidative stress may cause tissue to be more susceptible to oxidative damage and progression of disease in renal glomerolus (Brownlee, 2001; Yao et al., 2009).Histopathological evaluations on the diabetic kidney show expansion of mesangial matrix and uniform thickening of basement membranes in glomerulus and tubules (Ziyadeh and Wolf, 2008).

Since ancient times, plants have been a worthy source of medicine, which not only control hyperglycemia at low dosages but can also be taken for longer periods in contrast to synthetic hyperglycemic drugs (Grover et al., 2002). One of these plants is *Momordica charantia* (MC), also known as karalla, or bitter melon, which belongs to the cucurbitaceafamily, grows in tropical areas, including parts of the Amazon, east Africa, Asia, and the Caribbean, and is cultivated throughout South America as a food and medicine (Grover and Yadav, 2004).

The *Momordica charantia* (bitter melon) is a widely used plant in the traditional medicine for the treatment of diabetes mellitus (DM). It has been shown that *Momordica charantia* (Mc) has hypoglycemic effects on animals and humans, however, we don´t know if this effect is present in a chronic time and if the plant extract (stem and leaves) participates in the antihyperglycemic effect.

The *Momordica charantia* (MC) contains anti-hyperglycemic chemicals include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and steroids (Murakami et al., 2001; Erden et al., 2010). These chemicals are concentrated in fruits of the *Momordica charantia* (MC), therefore fruit of the MC has shown more pronounced anti-hyperglycemic activity (Grover and Yadav, 2004). Presence of antioxidants in the fruits and vegetables such as vitamin C, E, carotenoids, lycopenes and flavonoids are also important in prevent free radical injury (Semiz and Sen, 2007). Total flavonoid and phenol contents of *Momordica charantia* (MC) extract were analyzed and revealed that MC extract possess potent diphenylpicrylhydrazyl (DPPH) radical scavenging activity (Wu and Ng, 2008). Several studies have reported the anti-diabetic effects of MC on renal functional and histological changes in alloxan albino rats but only limited data is available on the anti-diabetic effects of MC on renal functional and histological changes in rats. **1.1. Aim**

The aim of this study is to investigate the effect of *Momordica charantia* leaf fractions on some serum electrolytes and renal biomarkers in alloxan-induced diabetic rats.

**1.1.0 Specific objectives**

i. To obtain fractions of *Mormodica charantia* leaf modified multi solvent serial extraction

ii. To evaluate the effect of *Mormodica charantia* leaf fractions on serum electrolytes and other renal function indices in alloxan induced diabetic albino rats.

****

**CHAPTER TWO**

**2.0 LITERATURE REVIEW**

*Momordica charantia*, known as bitter melon, bitter gourd, bitter squash, or balsam-pear, is a tropical and subtropical vine of the family Cucurbitaceae , widely grown in Asia, Africa, and the Caribbean for its edible fruit . Its many varieties differ substantially in the shape and bitterness of the fruit. It is sometimes known by names loaned from Asian languages. It is called Karela (singular) or Kareley (plural) in Hindi.

Bitter melon originated in India and was introduced into China in the 14th century. It is widely used in East Asian, South Asian, and Southeast Asian cuisine.



Fig 1: *Momordica charantia*

**2.1**. **SCIENTIFIC CLASSIFICATION**

Kingdom: Plantae

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Cucurbitales

Family: Cucurbitaceae

Genus: Momordica

Species: M. charantia

**2.2: GEOGRAPHICAL DISTRIBUTION OF MOMORDICA CHARANTIA**

According to Schaefer and Renner (2010) genus Momordica comprises 59 species distributed in the warm tropics, chiefly in Africa and with about 12 species in South-East Asia. *Momordica charanita* L. is the most widely cultivated species of Momordica. The generic name apparently derives from the Latin mordeo (to bite), perhaps a reference to the jagged edges of the seeds; charantia is from the ancient Greek for beautiful flower.

Momordica is in the subtribe Thladianthinae, tribe Joliffieae, subfamily Cucurbitoideae , Family Cucurbitaceae. There are five other cultivated Momordica species in addition to M. charantia. The other species are cultivatedin Asia and some parts of Africa ( Robinson and Decker-Walters, 1997 ).

Momordica is monophyletic and the genus can be divided into 11 clades. The Asiatic species falls under three sects. Dioecious species like M. cochinchinensis, M. dioica, M. sahyadrica, M. denticulata, M. denudata, M. clarkeana and M. subangulata grouped under the sect. Cochinchinensis, and monoecious species M. charantia and M. balsamina under the sect. Momordica and M. cymbalaria under the sect. Raphanocarpus (Schaefer and Renner, 2010 ; Behera et al., 2011 ). The monoecious species M charantia and M. balsamina produce edible fruits, and have been widely distributed as crops becoming naturalized throughout the tropics.

There are two botanical varieties viz.; M. charantia var. muricata (syn. var. abbreviata ) andM. charantia var. charantia, the former mostly wild and the latter cultivated. The wild variety (M. charantia var. muricata ) is considered as the progenitor of cultivated M. charantia var. (Walters and Decker-Walters, 1988 ).

**2.2.1. Habitat of *Momordica charantia***

M. Charantia grows under a very wide range of conditions throughout the tropics and subtropics. Its rapid growth and maturation allow it to colonize any area where there is sufficient short-term soil moisture, whilst it can also survive as a perennial in conditions of continuous soil moisture. It grows from sea level to over 1300 m ( Tjitrosoedirdjo, 1990 ), and in areas with annual rainfall as low as 480 mm. Minimum average annual temperatures may be as low as 12.5°C, though the plant is unlikely to thrive in such cold areas. It grows in soils with pH ranging from 4.3 to 8.7 (Holm et al., 1997 ).

**2.3 MEDICINAL PROPERTIES OF MOMORDICA CHARANTIA**

Bitter melon is traditionally known for its medicinal properties such as antidiabetic, anticancer, anti-inflammation, antivirus, and cholesterol lowering effects. It contains many phenolic compounds that may have the potential as antioxidant and antimutagen ( Budrat et al., 2008) (John et al., 2010). The fruit, stems, leaves and roots of bitter melon have all been used in traditional medicine to help treat ailments such as hyperlipidemia, digestive disorders, microbial infections and menstrual problems ( Yibchok et al., 2006).

(Ray et al., 2010). Showed that the extract of bitter melon modulates signal transduction pathways for inhibition of breast cancer cell growth and can be used as a dietary supplement for prevention of breast cancer.

**2.3.1 Preservation of pancreatic β cells and insulin secretion**

It was previously demonstrated by Jeewathayaparan et al. that oral administration of M. charantia could lead to the secretion of insulin from endocrine pancreatic β cell ( Jeewathayaparan et al., 1995) . This observation was further confirmed by (Ahmed et al., 2004) who investigated the effect of daily oral administration of M. charantia fruit juice and the distribution of α, β and δ cells in the pancreas of STZ-induced diabetic rats using immune histochemical methods.

The feeding of alcoholic extract from M. charantia showed definite improvement in the islets of Langerhans ( Singh et al., 2008).

**2.4. KIDNEY**

The kidneys are a pair of bean-shaped organs that are found in all vertebrates. The kidney is Located at the rear of the abdominal cavity in the retroperitoneal space, the kidneys receive blood from the paired renal arteries, and drain into the paired renal veins. Each kidney excretes urine into a ureter which empties into the bladder. They remove waste products from the body, maintaining the balance of electrolyte levels, and regulating the blood pressure.



**Fig 2. Structure of kidney**

**2.4.1. Kidney functions**

The Kidney maintains overall fluid balance regulation and helps in filtering minerals from blood, and equally filtering waste materials from food, medications, and toxic substances. It is a known fact that kidney function is compromised in uncontrolled diabetes mellitus. Glycosuria, a cardinal and diagnostic feature of diabetes imposes dehydration via glucose osmotic diuresis. This dehydration is accompanied with severe loss of electrolytes including sodium, potassium, calcium, chloride and phosphates (Gaw et al., 1995). Also in diabetes there is abnormally increased ketone body formation leading to ketonuria. Ketone bodies being moderately strong acids, on excretion carried along side with them buffer cations particularly alkaline cations (Na and K ) and also bicarbonates (Ramsey, 1986). Additional, substances otherwise not present in urine are excreted in urine including albumin (microalbuminuria) in diabetic condition. This undue passage distorts the repellent ability of structural polysaccharides (e.g., hyaluronic acids) whose function is to maintain the integrity of the kidney cells (Gaw et al., 1995), hence a distortion in the kidney basement membrane cell integrity. A combination of these factors and many more culminates in compromised kidney function in diabetes mellitus.

**2.4.2. Diseases of the Kidney**

Acute kidney injury (previously known as acute renal failure) – or AKI – usually occurs when the blood supply to the kidneys is suddenly interrupted or when the kidneys become overloaded with toxins.(Moore,*et al.,* 2012) (Ricci, *at al.,* 2012).

 Causes of acute kidney injury include accidents, injuries, or complications from surgeries in which the kidneys are deprived of normal blood flow for extended periods of time. Heart-bypass surgery is an example of one such procedure.

Drug overdoses, accidental or from chemical overloads of drugs such as antibiotics or chemotherapy, may also cause the onset of acute kidney injury. Unlike chronic kidney disease, however, the kidneys can often recover from acute kidney injury, allowing the patient to resume a normal life. People suffering from acute kidney injury require supportive treatment until their kidneys recovers function, and they often remain at increased risk of developing future kidney failure.

Among the accidental causes of renal failure is the crush syndrome, when large amounts of toxins are suddenly released in the blood circulation after a long compressed limb is suddenly relieved from the pressure obstructing the blood flow through its tissues, causing ischemia. The resulting overload can lead to the clogging and the destruction of the kidneys. It is a reperfusion injury that appears after the release of the crushing pressure. The mechanism is believed to be the release into the bloodstream of muscle breakdown products – notably myoglobin, potassium, and phosphorus that are the products of rhabdomyolysis (the breakdown of skeletal muscle damaged by ischemic conditions).

Kidney cancer: Renal cell carcinoma is the most common cancer affecting the kidney. Smoking is the most common cause of kidney cancer.

Chronic kidney disease (CKD): This is one of the diseases of the kidney, it has numerous causes. The most common causes of CKD are diabetes mellitus and long-term, uncontrolled hypertension. (Kes, *et al.,* 2013)Polycystic kidney disease is another well-known cause of CKD. The majority of people afflicted with polycystic kidney disease have a family history of the disease. Other genetic illnesses affect kidney function, as well as over-use of common drugs such as ibuprofen, and acetaminophen (paracetamol) can also cause chronic kidney disease. (Parnerger, et al., 1994).

Some infectious disease agents, such as hantavirus, can attack the kidneys, causing kidney failure. (Appel, *et al.,* 2012)

**2.5: SERUM ELECTROLYTES**

Electrolytes are the salts and metallic components that are dissolved within the blood serum (serum is the liquid portion of blood). Electrolytes carry an electrical charge, either negative or positive. The negatively charged electrolytes are balanced by the positively charged electrolytes.The major electrolytes are Na+, K+, Ca2+, Mg2+, Cl-, HCO3-, HPO42-, SO42- and lactate, as well as the trace elements. Both extracellular fluid (ECF) and intracellular fluid (ICF) contain electrolytes, a general term applied to bicarbonate and inorganic anions and cations.

**2.5.1 Functions of serum electrolytes**

 The role of electrolytes in the human body is manifold. Almost all metabolic processes depend on or are affected by electrolytes. Among the functions of the electrolytes are (i) maintenance of osmotic pressure and water distribution in the various body fluid compartments, (ii) maintenance of the proper pH, (iii) regulation of the proper function of the heart and other muscles, (iv) involvement in oxidation-reduction (electron transfer) reactions and (v) participation in catalysis as cofactors for enzymes. The abnormal levels of electrolytes maybe either the cause or the consequence of a variety of disorders,

**2.5.2: Clinical relevance of serum electrolytes**Sodium measurements are used in the diagnosis and treatment of aldosteronism (excessive secretion of the hormone aldosterone), diabetes insipidus (chronic excretion of large amounts of dilute urine, accompanied by extreme thirst), adrenal hypertension, Addison’s disease (caused by destruction of the adrenal glands), dehydration, inappropriate antidiuretic hormone secretion, or other diseases involving electrolyte imbalance. Potassium measurements are used to monitor electrolyte balance in the diagnosis and treatment of disease conditions characterized by low or high blood potassium levels. Chloride measurements are used in the diagnosis and treatment of electrolyte and metabolic disorders such as cystic fibrosis and diabetic acidosis.

**2.6. ALLOXAN INDUCED DIABETES**

Alloxan is a toxic glucose analogue, which selectively destroys insulin -producing cells in the pancreas (that is beta cells ) when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "alloxan diabetes") in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the Glucose transporter 2 (GLUT2) also known as solute carrier family 2 (facilitated glucose transport

**Fig 3: Structure of alloxan**

**2.6.1. Mechanism Of Action Of Alloxan**

A first transient hypoglycaemic phase of up to 30 min starts within minutes of alloxan injection. This short-lived hypoglycaemic response is the result of a transient stimulation of insulin secretion, as documented by an increase in the plasma insulin concentration. The underlying mechanism is a temporarily reduced consumption and increased availability of ATP caused by blockade of glucose phosphorylation through glucokinase inhibition. This initial transient hypoglycaemic phase is not observed in response to streptozotocin injection, since streptozotocin does not inhibit glucokinase. Morphological alterations are minimal during this phase.

The second phase starts with an increase in the blood glucose concentration, 1 h after administration of the toxins, and a decrease in plasma insulin. This first hyperglycaemic phase, which usually lasts 2–4 h, is caused by inhibition of insulin secretion leading to hypoinsulinaemia. During this phase the beta cells show the following morphological characteristics: intracellular vacuolisation, dilation of the rough endoplasmic reticulum, decreased Golgi area, reduced secretory granules and insulin content, and swollen mitochondria.

The third phase, again a hypoglycaemic phase, typically occurs 4–8 h after the injection of the toxins and lasts several hours. It may be so severe that it causes convulsions, and may even be fatal without glucose administration, in particular when liver glycogen stores are depleted through starvation. This severe transitional hypoglycaemia is produced by the flooding of the circulation with insulin as a result of toxin-induced secretory granule and cell membrane rupture. Pancreatectomy prevents this phase. In addition to the morphological changes seen in the first phase, the beta cell nuclei are pyknotic and show no TUNEL-positive staining; these changes are irreversible.

The fourth phase is the permanent diabetic hyperglycaemic phase. Morphologically, complete degranulation and loss of beta cell integrity is seen within 12–48 h. Non-beta cells remain intact, demonstrating the beta cell-selective character of the toxic action. Cell debris originating from the dying beta cells is removed by non-activated scavenger macrophages.

Thus, injections of alloxan and streptozotocin principally induce the same blood glucose and plasma insulin responses and cause an insulin-dependent type 1-like diabetes syndrome. All of the described morphological features of beta cell destruction are characteristic of necrotic cell death (Lenzen S 2017). This mechanism is clearly at variance with that which underlies autoimmune type 1 diabetes, both in humans and rodent models of the disease, where beta cell demise is the result of apoptotic cell death without leakage of insulin from ruptured secretory granules ( Lenzen S 2017).

**2.6.2. Effects Of Alloxan**

Alloxan has two distinct pathological effects: it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and it causes a state of insulin-dependent diabetes through its ability to induce ROS formation, resulting in the selective necrosis of beta cells. These two effects can be assigned to the specific chemical properties of alloxan, the common denominator being selective cellular uptake and accumulation of alloxan by the beta cell.

**CHAPTER THREE**

**MATERIALS AND METHODS**

**3.1** **Materials**

Glucose strips

Whatmann filter paper

Animal cages

Insulin syringes

Spatula

Razor blade

Injection bottles

Latex gloves

 **3.1.1.** **Chemicals and reagents**

|  |  |
| --- | --- |
| Methanol | JHD methanol AR. China |
| Chloroform | BDH laboratory reagents  |
| Acetylacetate | BDH Laboratory reagents  |
| Hexane | BDH AnalaR |
| Alloxan | Qualikems India |
| Glibenclamide | Hovif Bhd. Malaysia  |

Potassium reagent set ( TECO DIAGNOSTIC )

Urea Nitrogen reagent set. (colorimetric method)( TECO DIAGNOSTIC).

Chloride reagent set (TECO DIAGNOSTIC)

Sodium reagent set ( TECO DIAGNOSTIC)

Creatinine reagent set ( TECO DIAGNOSTIC)

**3.1.2 Equipments/Instruments**

|  |  |
| --- | --- |
| Glucometer | Accuchek advantage II |
| Water bath | Model HH. W21. Cr42II |
| Spectrophotometer | SpectrumLap23A |
| Centrifuge | Model 80-2 |
| Incubator |  |
| Weighing balance | Model no. YP-502N |
| Refrigerator | Kelvinator, Germany  |

**3.2 THE PLANT COLLECTION**

Leaves of Momordica charantia were collected in May 2018 from Orba in Nsukka Local Government Area and Ebony state. The taxonomical identification of the plant was done by Prof C. U. Okeke, at the Habarium, school of biological sciences. Botany department, Nnamdi Azikiwe University. Harvesting was done on a dry day.

**3.2.1.1 Preparation of Leave Extract and Fractions**

The plant leaves were quickly dried under a shade at a temperature of below 30°C in a dust-damp-free environment that was free of contamination for 5 days.

The dry leaves were then pulverized into fine powder using Grinding Machine. The dried plant was weighed to be 450g. The 450g of dried plant material was poured into a small plastic bucket and 2litres of Methanol was added to it and starred. It was left for 48hrs under constant starring. After the 48hrs of soaking the plant. It was extracted using and was further filtered using whatmann filter paper. It was dried using water bath with the temperature under 45 to 60°C for 5days. The dried extract was weighed to be 25.86g. The 25.86g of the crude extract was subjected to fractionation using 200ml of hexane to 100ml of distilled water. The hexane layer was collected as the hexane fraction while the aqueous layer was collected and further extracted with 200ml of chloroform and 100ml of distilled water. The chloroform layer was considered as the chloroform fraction while the aqueous layer was subjected to ethyl acetate and were, in the ratio of 2:1. The ethyl acetate layer was obtained as the ethyl acetate fraction while the aqueous layer was further subjected to methanol extraction. The methanol soluble part was considered as the methanol fraction after sieving while the insoluble part was considered as the aqueous fraction. A total of 5 fractions ( Hexane, chloroform, ethyl acetate, methanol and aqueous fractions) were obtained.

 **Crude methanol extract**

 **Hexane and water 2:1**

 **Hexane layer. Aqueous layer**

**Hexane fraction. Chloroform & water 2:1.**

 **Chloroform layer. Aqueous layer.**

 **Chloroform fraction. Et. Ac and water 2:1.**

 **Ethyl acetate layer. Aqueous layer.**

 **Ethyl acetate fraction. Methanol + residue.**

 **Methanol soluble part. Methanol insoluble part.**

 **Methanol fraction. Aqueous fraction**

**3.2.2 Acquisition, Care and Maintenance of Experimental Animals**

Forty albino rats were used for the study. They were obtained from the University of Nigeria Nsukka. The rats were all put into two different cages and were fed with grower and water for 7days. On the 8th day the rats were weighed and separated into 8 different cages.

**3.2.2.1 Induction of Alloxan in Rats**

Normal saline was first of all prepared using 0.9g of NACL in 100ml of distilled water. After the preparation of normal saline, 1g of alloxan was dissolved in 50g of Normal saline. Before the induction of the alloxan in the rats, the glucose level of the rats was determined using Accu check method.

Thirty five (35) fasted rats were intraperitoneally injected with 100 mg/kg of alloxan and the remaining five were used as the positive control. After 48 to 72hrs of the induction the rats glucose were checked and the levels was observed to have increased and set for treatment with the plant extracts.

**3.3.3 PREPARATION OF EXTRACT FOR ADMINISTRATION**

All the additives used in formulation were natural plant products and a standard drug, and the form of drug administration was syrup, which consisted of a concentrated dissolution of normal saline and plant extract.

**3.3.3.1 Preparation Of Glibenclamide Stock Solution**

Glibenclamide (standard drug) was smashed and 0.5g was suspended into 30ml of normal saline. The mixture was kept in a cool temperature.

**3.3.3.2 Preparation Of Ethyl Acetate Fraction Stock Solution**

The ethyl acetate fraction was dried and weighed 2.34g. It was dissolved in 100ml of normal saline. The mixture was kept in cool temperature.

**3.3.3.3 Preparation Of Hexane Fraction Stock Solution**

The hexane fraction was weighed after drying and it weighed 2.66g. It was further dissolved in 100ml of normal saline. The moisture was kept under cool temperature.

**3.3.3.4 Preparation Of Methanol Fraction Stock Solution**

The methanol fraction was weighed after drying and it weighed 1.87g. It was further dissolved in 100ml of normal saline. The mixture was kept under cool temperature.

**3.3.3.5 Preparation Of Chloroform Fraction Stock Solution**

The chloroform fraction was weighed after drying with water bath and it weighed 11.16g. It was further dissolved in 100ml of normal saline. The mixture was kept under cool temperature.

**3.3.3.6 Preparation Of Aqueous Stock Solution**

The aqueous fraction was weighed after drying with water bath, and it weighed 1.82g. It was suspended in 100ml of normal saline. The solution was kept in a cool temperature.

**3.4 ADMINISTRATION**

Each group of animals were administered with the effective doses once a day. The extracts were administered in their syrup form. 2ml of syringes were used for intra-gastric oral intubatiion was used for administration to the rats.

**3.5 EXPERIMENTAL DESIGN**

Thirty five (35) diabetic rats and 5 normal rats were divided into 8groups of 5rats each. Group Group A consisted of diabetic rats treated with Glibenclamide 200mg/kg

Group B consisted of diabetic rats treated with Hexane fraction 200mg/kg

Group C consisted of diabetic rats treated with Aqueous fraction 200mg/kg

Group D diabetic rats treated with Methanol fraction 200mg/kg

Group E diabetic rats treated with Ethyl acetate fraction 200mg/kg

Group F diabetic rats treated with Chloroform fraction 200mg/kg

Group G diabetic rats untreated received water and food negative control

Group H. Not diabetic, normal control

**3.5.1 Experimental Procedure**

Each rats were treated with the different extracts depending on the group they fell into. And they were treated through oral intubation for 21days under close watch. After which their glucose level were determined.

**3.6 BLOOD COLLECTION**

After 2weeks of treatment the 7groups of Alloxan-induced diabetic rats were sacrificed. They were put inside a tight container that contained cotton wool soaked with chloroform, which made them became unconscious, before blood was taking from their hearts.

**3.7 DETERMINATION OF THE DIFFERENT PARAMETERS**

**3.7.1 Principle And Procedure For Testing Urea Nitrogen (Bun)**

The BUN colorimetric procedure is a modification of the Berthelot reaction. Urea is converted to ammonium by the use of urease. Ammonium ion then reacts with a mixture of salicylate, sodium nitroprusside, and hypochlorite to yield a blue-green chromophore. The intensity of color formed is proportional to the urea concentration in the sample. Expected values 7-23 mg/dl

**Procedure For Testing Urea Nitrogen (BUN)**

1.5ml of BUN enzyme was pipetted into labeled test tubes (sample and standard test tubes) and allowed to equilibrate at room temperature.0.01ml of each of the samples were added to the respective test tubes and deionied water was added to the reagent blank.

The samples were incubated for 5minutes at 37⁰C.

After incubation, 0.5ml of BUN color developer was added to the samples and mixed gentlely.

The samples were incubated for another minutes at 37⁰C, after which the wavelength of the spectrophotometer was set at 630nm, and it was zeroed with blank reagent and the absorbance of the samples were recorded.

**Calculations** Absorbance of sample x concentration of standard ₌ urea nitrogen (mg/dl)

 Absorbance of standard

**3.7.2 Principle and Procedure For Testing Sodium**

The present method is based on modifications of those first described by Maruna.( Maruna, RFL. 1958) and Trinder ( Trinder, P. 1952) in which sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely aa the concentration of sodium in the test specimen. It ranges from 135 - 155 m£q/L.

 **Procedure For Testing Sodium**

Test tube were labeled as follows: blank, standard and samples

1.0ml of filtrate reagent was added to all the test tubes

50µl of samples were pipetted into all the test tubes except the blank that was added 50µ of distilled water.

All the test tubs were vigorously shaken and mixed continuously for 3minutes.

The tubes were centrifuged for 10minutes at 1500rpm and the supernatant fluid was tested below

Test tubes were labeled corresponding to the above filtrate tubes

1.0ml of the acid reagent was added to all tubes

50µ of the supernatant was added to the respective tubes.

 50µ of color reagent was added to all tubes and mixed.

The spectrophometer was zeroed with distilled water at 550nm and the absorbances of the samples were recorded.

Absorbance of blank − absorbance of sample x concentration of standard ₌ concentration of sample

Absorbance of blank − absorbance of standard

**3.7.3. Principle and Procedure For Testing Creatinine**

Creatinine reacts with picricvacid in alkaline conditions to form a color complex, which absorbs at 510 nm. The rate of formation of color is proportional to the creatinine concentration in the sample. In the endpoint method, the difference in absorbance measurements after color formation yields a creatinine value corrected for interfering substances.

**Procedure For Creatinine Reagent Analysis**

Equal volumes of Creatinine Picric Acid Reagent and Creatinine Buffer Reagent were mixed together and stirred. The test tubes were labeled as Reagent Blank, Standard, and Samples. 3.0ml of the working reagent prepared above was pipette into the respective test tubes. Thereafter, 0.1ml of the samples was transferred to their respective tubes and distilled water to the reagent blank tube. The mixture was mixed gently and then placed in a heating water bath at 37oC for 15minutes. Then a Spectrophotometer was set at absorbance with wavelength of 510nm to read the absorbance of the samples against the blank (the blank was used to standardize the spectrophotometer before the absorbance of the samples was measured).

The Creatinine values of the samples were determined by comparing its absorbance change with that of the standard.Calculations:

Creatinine vcalue (mg/dl) = Abs. (Sample) x Concentration of Standard

Abs. (Standard)

Where: Abs= Absorbance

**3.7.4 Principle And Procedure For Testing Potassium**

The amount of potassium is determined by using sodium tetra phenol boron in a specifically prepared mixture to produce a colloidal suspension. Terri, A.E., et al. (1958). The turbidity of which is proportional to potassium concentration in the range of 2 - 7 m£q/L.

**Procedure For Potassium Analysis**

The method of Henry, R.F. et. al. (1974) was applied in this analysis.

 Test tubes were labeled as blank, standard and samples.

Then 0.1ml of potassium reagent was added to all tubes followed by addition of 0.01ml of the samples to the respective tubes.

The solutions were mixed and let to sit at room temperature for 3mins. After the 3mins, the absorbance of the samples were read at 500nm wavelength of the spectrophotometer using the reagent blank to standardized the spectrophotometer.

**Calculations**: Potassium Conc.(mEq/L)

Absorbance of sample x concentration of standard (mEq/L)

Absorbance of standard

**3.7.5. Principle And Procedure For Testing Chloride**

Chloride ions form a soluble, non-ionized compound, with mercuric ions and will displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions react with ferric ions to form a color complex that absorbs light at 480 nm. The intensity of the color produced is directly proportional to the chloride concentration.

**Procedure For The Test**

Test tubes were labeled as blank, standard and samples.

Then 0.5ml of chloride reagent was added to all tubes followed by addition of 0.01ml of the samples to the respective tubes.

The solutions were mixed and incubated for 5mins. After the 5mins, the absorbance of the samples were read at 450nm wavelength of the spectrophotometer using the reagent blank to standardized the spectrophotometer.

**Calculations:** Potassium Conc.(mEq/L)

Absorbance of sample x concentration of standard (mEq/L)

Absorbance of standard

**3.8 STATISTICAL ANALYSIS**

The data obtained were subjected to statistical analysis. Means were complained using analysis of variance ( ANOVA) with the statistical tool SPSS. values having p<0.05 were considered significant.

**CHAPTER FOUR**

**RESULTS AND ANALYSIS**



**FIG. 4.1: Shows the bar chart of statistical analysis of Urea level for the diabetic treated and untrated animals in the study groups.**

**Bullets:**

Group one= Diabetic group treated with standard anti-diabetic drug (Glibenclamide)

Group two= Diabetic group treated with hexane crude fraction of *Mormodica charantia*

Group three= Diabetic group treated with aqueous crude fraction of *Mormodica charantia*

Group four= Diabetic group treated with methanol crude fraction of *Mormodica charantia*

Group five= Diabetic group treated with ethylacetate crude fraction of *Mormodica charantia*

Group six= Diabetic group treated with chloroform crude fraction of *Mormodica charantia*

Group seven= Diabetic untreated group (-ve control group)

Group eight= Non- diabetic (+ve control group)



**FIG. 4.2: Shows the bar chart of statistical analysis of Creatinine level for the diabetic treated and untrated animals in the study groups.**



**FIG. 4.3: Shows the bar chart of statistical analysis of Potassium level for the diabetic treated and untrated animals in the study groups.**

Effectof *momordica charantia* leaf extract on sodium level the result showed a significant (p>0.05) increase in group1 of the treated with standard drug and group2 of treated with leaf extract compared to that of the normal group, a non-significant (p>0.05) increase in group6.



**FIG.4.4:Shows the barchart of statistical analysis of Chloride level for the diabetic treated and untrated animals in the study groups.**



**FIG. 4.5: Shows the bar chart of statistical analysis of Sodium level for the diabetic treated and untrated animals in the study groups.**

 **PERCENTAGE YIELD OF THE FRACTIONS**

|  |  |  |
| --- | --- | --- |
| **Plant extract** | **Weight**  | **Percentage yield (%)** |
| Methanol | 1.87 | 0.42 |
| Hexane | 2.66 | 0.59 |
| Chloroform | 11.16 | 2.48 |
| Ethylacetate | 2.34 | 0.52 |
| Aqueous  | 1.89 | 0.42 |

**CHAPTER FIVE**

**DISCUSSION, CONCLUSION AND PROSPECTIVE**

**5.1 Discussion**

Diabetes is the World’s largest growing metabolic disorder, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases (Baily and Flat, 1986). Kidney is an organ that regulates water and electrolytes. Also, high blood sugar can increase plasma osmolarity thereby, drifting water movement from the intracellular to the extracellular spaces and alter the distribution of electrolytes in the body. The kidney has a vital organ in the body which helps in secrating the waste of the body in the form of urine which consists of creatinine, urea BUN (Blood Urea Nitrogen).

The current study investigated the effects of Momordica charantia fractions on renal function indices and serum electrolytes of alloxan induced diabetic rats. The results herein indicate that in renal indices analysis ( Creatinine and Urea) there was a mass significant decrease (P>0.05) in test groups treated with the extract and the standard drug when compared to the diabetic untreated groups.

In the figures showing the result analysis of serum electrolytes( potassium, chloride and sodium), indicate that there was a significant decrease (P>0.05) in all the groups treated with the extract and the group treated with standard drug when compared with the untreated diabetic group.

 Hyperglycemia leads to hyperosmolarity and reduction of intracellular water (Collins, 2007). Sodium is the most abundant extracellular ion, and it plays an important role in muscle contraction. Similarly, potassium, an abundant intracellular ion, plays a vital role in muscle contraction. The electrolyte derangement resulting from the reduced serum level of sodium, potassium and Chloride as seen in this study, thus provides evidence that the use of *Momordica charantia* leave fractions can reduced the risk of electrolytes imbalance.

**5.2 Conclusion** The results of this present research has shown that the leave of  *Momordica charantia*  did not restrict the high loss of serum electrolytes associated with diabetes .Hence care should be taken in the use of the plant medicinal purposes.

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**APPENDIX**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Weight in gram** |  | **Glucose level before alloxan** | **Alloxan in gram** | **Alloxan in ml** | **Alloxan in microlitre** | **2 days after induction** | **3 days after induction** |
| **Group A** | **Glibenclamide** |
|  | 121.1 | No mark  | 90mg/dl | 0.012 | 0.6 | 24ul | 103 | 211 |
|  | 119.47 | Tail | 97mg/dl | 0.012 | 0.6 | 24ul | 115 | 230 |
|  | 93.28 | Head | 77mg/dl | 0.010 | 0.5 | 20ul | 111 | 221 |
|  | 138.37 | Stomach  | 90mg/dl | 0.012 | 0.6 | 24ul | 114 | 236 |
|  | 141.44 | Back | 96mg/dl | 0.014 | 0.7 | 28ul | 115 | 241 |
| **Group B** | **Hexane**  |
|  | 117.95 | No mark | 211mg/dl | 0.012 | 0.6 | 24ul | 213 | 303 |
|  | 87.47 | Head | 72 mg/dl | 0.009 | 0.45 | 18ul | 101 | 188 |
|  | 96.7 | Back | 73mg/dl | 0.01 | 0.5 | 20ul | 98 | 203 |
|  | 116.57 | Tail  | 73mg/dl | 0.012 | 0.6 | 24ul | 113 | 233 |
|  | 147.45 | Stomach  | 73mg/dl | 0.015 | 0.75 | 30ul | 99 | 199 |
| **Group C** | **Aqueous Fraction**  |
|  | 143.51 | No mark | 59 | 0.014 | 0.7 | 28ul | 89 | 189 |
|  | 176.07 | Tail | 93 | 0.017 | 0.85 | 34ul | 111 | 168 |
|  | 139.57 | Stomach  | 180 | 0.14 | 0.7 | 28ul | 199 | 285 |
|  | 120.75 | Back  | 106 | 0.012 | 0.6 | 24ul | 201 | 300 |
|  | 124.95 | Head  | 103 | 0.01 | 0.5 | 20ul | 198 | 238 |
| **Group D**  | **Methanol**  |
|  | 128.65 | No mark  | 76 | 0.013 | 0.65 | 26ul |  |  |
|  | 163.72 | Tail  | 88 | 0.02 | 1.0 | 40ul |  |  |
|  | 115.45 | Back  | 93 | 0.012 | 0.6 | 24ul |  |  |
|  | 134.31 | Stomach  | 85 | 0.013 | 0.65 | 26ul |  |  |
|  | 132.62 | Head  | 97 | 0.013 | 0.65 | 26ul |  |  |
| **Group E** | **Et. Ac** |
|  | 133.57 | No mark | 93 | 0.013 | 0.65 | 26ul | 122 | 297 |
|  | 79.75 | Stomach  | 74 | 0.008 | 0.4 | 16ul | 97 | 173 |
|  | 129.57 | Tail  | 111 | 0.013 | 0.65 | 26ul | 103 | 333 |
|  | 120.25 | Back  | 103 | 0.012 | 0.6 | 24ul | 117 | 252 |
|  | 156.57 | Head  | 55 | 0.02 | 1.0 | 40ul | 88 | 151 |
| **Group F** | **Chlorofoam**  |
|  | 165.84 | Back  | 80 | 0.02 | 1.0 | 40ul | 115 | 205 |
|  | 164.45 | No mark | 85 | 0.02 | 1.0 | 40ul | 119 | 265 |
|  | 102.03 | Head | 157 | 0.010 | 0.5 | 20ul | 105 | 232 |
|  | 131.50 | Stomach  | 84 | 0.013 | 0.65 | 26ul | 110 | 209 |
|  | 137.00 | Tail  | 77 | 0.014 | 0.7 | 28ul | 113 | 213 |
| **Group G** | **Alloxan No Treatment** |
|  | 129.28 | Tail  | 87 | 0.013 | 0.65 | 26ul | 106 | 299 |
|  | 157.49 | Back  | 93 | 0.016 | 0.8 | 32ul | 128 | 334 |
|  | 103.28 | Head  | 90 | 0.010 | 0.5 | 20ul | 115 | 241 |
|  | 98.81 | Stomach  | 78 | 0.01 | 0.5 | 20ul | 111 | 190 |
|  | 144.85 | No mark  | 79 | 0.014 | 0.7 | 28ul | 98 | 234 |
| **Group H** | **No Alloxan**  |
|  | 152.37 | Tail  | 79mg/dl |  |  |  |  |  |
|  | 151.01 | Head  | 98mg/dl |  |  |  |  |  |
|  | 172.61 | No mark  | 111mg/dl |  |  |  |  |  |
|  | 147.22 | Back  | 96mg/dl |  |  |  |  |  |
|  | 140.25 | Stomach  | 93mg/dl |  |  |  |  |  |

**Administration of Alloxan**

100mg/kg pose

**Group A**

**121 gram No mark rat**

1000 g = 100 mg

121 g = ?

Mg = $\frac{121 ×100}{1000}$

$$\frac{12.1}{1000}$$

= 0.012g

**119.41 g Tail**

1000 g = 100 mg

119.41 g = ?

Mg = $\frac{119.41 ×100}{1000}$

$$\frac{11.9}{1000}$$

= 0.0119g $≅0.012g$

**93g Head**

1000 g = 100 mg

93g = ?

Mg = $\frac{93 ×100}{1000}$

$$\frac{9.3}{1000}$$

= 0.009g $≅0.01g$

**93g Stomach**

1000 g = 100 mg

138.37g = ?

Mg = $\frac{138.37 ×100}{1000}$

$$\frac{13.837}{1000}$$

= 0.012g

**141.44g Head**

1000 g = 100 mg

141g = ?

Mg = $\frac{141.44 ×100}{1000}$

$$\frac{14.144}{1000}$$

= 0.014g

**Group B**

**117.95 gram No mark rat**

1000 g = 100 mg

117.95 g = ?

Mg = $\frac{117.95 ×100}{1000}$

$$\frac{11.7}{1000}$$

= 0.012g

**87.47 g Head**

1000 g = 100 mg

87.47 g = ?

Mg = $\frac{87.47 ×100}{1000}$

$$\frac{8.7}{1000}$$

= 0.009g

**96.76g Back**

1000 g = 100 mg

96.76g = ?

Mg = $\frac{96.76 ×100}{1000}$

$$\frac{9.6}{1000}$$

= $0.01g$

**116.57 Tail**

1000 g = 100 mg

116.57g = ?

Mg = $\frac{116.57 ×100}{1000}$

$$\frac{11.6}{1000}$$

= 0.012g

**147.45g Stomach**

1000 g = 100 mg

147.45g = ?

Mg = $\frac{147.45 ×100}{1000}$

$$\frac{14.7}{1000}$$

= 0.015g

**Group C**

**143.51 gram No mark rat**

1000 g = 100 mg

143.51 g = ?

Mg = $\frac{143.51 ×100}{1000}$

$$\frac{14.3}{1000}$$

= 0.014g

**176.07 Tail**

1000 g = 100 mg

176.07 g = ?

Mg = $\frac{176.07 ×100}{1000}$

$$\frac{17.6}{1000}$$

= 0.017g

**139.57g Stomach**

1000 g = 100 mg

139.57g = ?

Mg = $\frac{139.57 ×100}{1000}$

$$\frac{13.9}{1000}$$

= $0.14g$

**120.75 Back**

1000 g = 100 mg

120.75g = ?

Mg = $\frac{120.75 ×100}{1000}$

$$\frac{12.0}{1000}$$

= 0.012g

**124.95g Head**

1000 g = 100 mg

124.95g = ?

Mg = $\frac{124.95 ×100}{1000}$

$$\frac{12.4}{1000}$$

= 0.01g

**Group D**

**128.65 gram No mark rat**

1000 g = 100 mg

128.65 g = ?

Mg = $\frac{128.65 ×100}{1000}$

$$\frac{12.8}{1000}$$

= 0.013g

**163.72 g Tail**

1000 g = 100 mg

163.72 g = ?

Mg = $\frac{163.72 ×100}{1000}$

$$\frac{16.4}{1000}$$

= 0.02g

**115.43g Back**

1000 g = 100 mg

115.43g = ?

Mg = $\frac{115.43 ×100}{1000}$

$$\frac{11.5}{1000}$$

= $0.012g$

**134.31 Stomach**

1000 g = 100 mg

134.31g = ?

Mg = $\frac{134.31 ×100}{1000}$

$$\frac{13.4}{1000}$$

= 0.013g

**147.45g Head**

1000 g = 100 mg

132.62g = ?

Mg = $\frac{132.62 ×100}{1000}$

$$\frac{13.3}{1000}$$

= 0.013g

**Group E**

**133.57 gram No mark rat**

1000 g = 100 mg

133.57 g = ?

Mg = $\frac{133.57 ×100}{1000}$

$$\frac{13.3}{1000}$$

= 0.013g

**79.75 g Stomach**

1000 g = 100 mg

79.75 g = ?

Mg = $\frac{79.75 ×100}{1000}$

$$\frac{7.9}{1000}$$

= 0.008g

**129.57g Tail**

1000 g = 100 mg

129.57g = ?

Mg = $\frac{129.57 ×100}{1000}$

$$\frac{12.9}{1000}$$

= $0.013g$

**120.25 Back**

1000 g = 100 mg

120.25 g = ?

Mg = $\frac{120.25 ×100}{1000}$

$$\frac{12.1}{1000}$$

= 0.012g

**156.57 Head**

1000 g = 100 mg

156.57 = ?

Mg = $\frac{156.57 ×100}{1000}$

$$\frac{15.7}{1000}$$

= 0.02g

**Group F**

**165.84 Back**

1000 g = 100 mg

165.84 g = ?

Mg = $\frac{165.84 ×100}{1000}$

$$\frac{16.6}{1000}$$

= 0.02g

**No mark= 164.45**

1000 g = 100 mg

164.45 g = ?

Mg = $\frac{164.45 ×100}{1000}$

$$\frac{16.5}{1000}$$

= 0.02g

**Head =102.3**

1000 g = 100 mg

102.3g = ?

Mg = $\frac{102.3 ×100}{1000}$

$$\frac{10.3}{1000}$$

= $0.01g$

**Stomach 131.50**

1000 g = 100 mg

131.50 g = ?

Mg = $\frac{131.50 ×100}{1000}$

$$\frac{13.2}{1000}$$

= 0.013g

**Tail 137.00**

1000 g = 100 mg

137.00 = ?

Mg = $\frac{137.00 ×100}{1000}$

$$\frac{13.7}{1000}$$

= 0.014g