

Effects of Ethanolic Extract of *Telfairia Occidentalis* on Glucose Level and Renal Function Parameters in Streptozotocin Induced Diabetic Rats

Fikayo Noah Adegboyega^{1*}, Gift Adebola Amoo²

^{1,2}Department of Biochemistry, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomosho, Nigeria.

***Corresponding Author**

E-mail Id:-adegboyegafikayo@gmail.com

ABSTRACT

One of the leading challenges to public health globally is diabetes mellitus. Diabetes mellitus is a chronic condition caused by irregular high level of glucose in the blood. However, therapeutic option mostly adopted is the use of natural plant derivatives. The potential protective effect of *Telfairia occidentalis* was evaluated in blood serum and kidney following Streptozotocin-induced toxicity in male Wistar rats. Twenty-five male albino rats of Wistar strain were randomly allocated into five groups: Group A: Streptozotocin – induced diabetic rats (animals injected with 50mg/kg b. wt. of Streptozotocin intravenously), and treated with low dose of ethanolic leaf extract of *Telfairia occidentalis* (200mg/kg b. wt.) for eight days, Group B: Streptozotocin – induced diabetic rats, and then treated with high dose of ethanolic leaf extract of *Telfairia occidentalis* (400mg/kg b. wt.) for eight days, Group C: Streptozotocin – induced diabetic rats treated with Metformin (1.43mg/kg b. wt.) for eight days, Group D: Untreated Streptozotocin – induced diabetic rats, Group E: Control (animals received distilled water only for eight days). Blood glucose concentration was determined at every 48 hours for 8 days. Other biochemical parameters such as sodium and potassium concentration, total protein (TP) concentration, albumin concentration, creatinine and urea concentration were also determined. Streptozotocin induced diabetic rats showed significant ($p<0.05$) increase in blood glucose level. Treatment with ethanolic leaf extract of *Telfairia occidentalis* showed a statistically significant ($p<0.05$) dose dependent reduction in blood glucose levels with a steep descent seen at higher dose. Streptozotocin significantly increased ($P<0.05$) the serum level of sodium, potassium, creatinine and urea, while it decreased the serum total protein and albumin concentration. Treatment with ethanolic leaf extract of *Telfairia occidentalis* significantly ($p<0.05$) decreased serum level of sodium, potassium, creatinine and urea, while it increased the serum total protein and albumin concentration. The results of this study showed that ethanolic leaf extract of *Telfairia occidentalis* is good antihyperglycaemic agent in lowering the blood glucose level. Hypernatremia and hyperkalaemia (diabetic ketoacidosis) caused by Streptozotocin are also lowered through the use of ethanolic leaf extract of *Telfairia occidentalis*. This may therefore suggest the effect of ethanolic leaf extract of *Telfairia occidentalis* in protecting renal damage in streptozotocin induced diabetes. Therefore, the protective mechanism of *Telfairia occidentalis* might be due to its free radical scavenging potential and antidiabetic property.

Keywords:-streptozotocin, *telfairia occidentalis*, metformin, diabetes mellitus, therapy, phytochemistry, hypernatremia and hyperkalaemia

INTRODUCTION

Abnormality or derangement in blood

could be traced to presence of many metabolites, one of such is high level of

glucose that is associated with diabetes mellitus. Diabetes mellitus is globally believed to be one of the leading challenges in public health society caused by the abnormality of carbohydrate metabolism which is directly linked to low blood insulin level or inconsiderateness of target organs to insulin [1]. If this disease is left unattended to, it can elicit a number of complications such as renal disease, nerve disease, blindness, cardiovascular disorder, etc[1]. It has affected over 245 million people worldwide with the figure keeps increasing daily [20]. The way it emanating speed is experienced in Nigeria, is exponentially considering its effect on the socioeconomic growth over the past few years which has led to a sedentary lifestyle of workers and occupants living in a densely populated places or urban regions [23]. Previous studies done by Al-Daghri *et al.*[2] reviewed the epidemiological increase in the prevalence of diabetes mellitus during the past decades. The algebraic estimation figure in prevalence of diabetes mellitus is expected to be double by 2030 (Wild *et al.*, 2004).

Diabetes mellitus is medically and physiologically referred to as silent killer which is taking its toll on inhabitants of world[25]. In providing remedy, adoption of antidiabetic therapies including insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and glinides as suggested by James *et al.*[13] are used. However the results garnered from these therapies were with adverse effects, there is therefore the need for agents that will not only be more effective but should be relatively safe to lower the blood glucose. Another treatment procedure earlier adopted by James *et al.*[13] was the glucose reduction to sorbitol by a potent Aldol reductase enzyme via its catalytic pathway. The culmination of sorbitol (reduced glucose) also contributes to various complications of diabetes mellitus including neuropathy, nephropathy and

cataract [13].

The present study investigates one of the therapeutic processes that could be explored in treating diabetes mellitus and its complications. Natural plant metabolites are mostly used to treat diabetes mellitus because of their richness in flavonoids – key external antioxidant sources that can mop up and detoxify the free radicals generated during cell metabolism.

LITERATURE REVIEW

Streptozotocin (STZ)

Streptozotocin is a regular diabetogenic agent that induces perpetual diabetes in animal models by causing disruption of pancreatic β -cells and thus stops insulin production. Its β -cell toxicity is by carbamoylation of proteins, alkylation of DNA, release of free radicals – reactive oxygen species (ROS) and reactive nitrogen species (RNS) alongside with inhibition of O-GlcNAcse (glycoside hydrolase) (Dolan, 1997). ROS and RNS generated during decomposition and metabolism of STZ diminish the activities of mitochondrial enzymes and inhibit O-GlcNAcse a glycoside hydrolase causing total energy levels of β -cell and suppressing biological function of proteins of islet cells[6]. The suppression of biological function is the reason for low or no output of insulin.

Reports generated by Haiderkaza *et al.*, (2011) about action of streptozotocin reveal tissue-specific increase in oxidative stress with persistent increase in the generation of free radicals in the streptozotocin treated subjects. The Free radicals generated include reactive oxygen and nitrogen species (ROS and RNS) like superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2) and Peroxynitrite ($ONOO^-$) (Haiderkaza *et al.*, 2011). Alireza *et al.*[3] also work on streptozotocin and they demonstrated that

hyperglycaemia in STZ induced diabetes is associated with increased formation of reactive oxygen species (ROS) and oxidative damage to tissue compounds [3].

Diabetes Mellitus

Diabetes mellitus (DM) is a multifaceted syndrome characterized biochemically by hyperglycaemic and altered glucose metabolism. It is associated pathologically with specific vascular complications [24]. In its typical form, common forms of diabetes mellitus are: Type 1 or insulin-dependent diabetes mellitus (IDDM) and Type 2 or non-insulin-dependent diabetes mellitus (NIDDM). Type 1 DM results from either of the interaction of genetic or environmental or immunologic elements that lead to disorder in pancreatic beta cells. It also emanates as a result of insulin deficiency[8]. The study according to Eisenbarth[8] agrees that Type 1 DM also results from autoimmune beta cell destruction and some individuals who have the clinical phenotype of type 1 DM lack immunologic markers which is an indicative of an autoimmune destruction involving the beta cells[8].

Insulin resistance and derangement in insulin secretion are central to the development of type 2 DM[10]. Insulin resistance is the ineffectiveness of insulin to act on the target tissues is a prominent feature of type 2 DM. Type 2 DM has a strong genetic relation other than environmental factors (such as nutrition, physical activities and obesity). It is therefore said to be polygenic and multifactorial disease [4]. In type 2 Diabetes Mellitus, insulin resistance in the liver reveals the failure of hyperinsulinemia to retard the gluconeogenesis process, thus decreases glycogen storage by the liver in the postprandial level. Elevation in hepatic glucose synthesis occurs early in the diabetic state as a result of insulin resistance in adipose tissue and obesity, free fatty acid (FFA) flux from adipocytes is increased, leading to increased lipid

synthesis in hepatocytes.

Drugs Used in Treatment

Most substances that have the affinity to lower the glucose level in the blood are said to be anti-diabetic medications adopted in diabetic therapy. Majority of these medications are administered orally, and get metabolized by liver via efficient liver metabolism, except for insulin, pramlintide and exenatide that are administered intravenously. The anti – diabetic drugs administered orally are sometimes called oral hypoglycemic agents or oral antihyperglycaemic agents. However their selection depends on the nature of the diabetes, age and condition of the person, as well as other hovering factors[10].

Metformin as an Anti-Hyperglycaemic

Metformin is an oral drug used as antihyperglycaemic agent widely accepted in treating type 2 diabetes. Structurally, it composes of a biguanide supplanted by phenformin, another biguanide. It was firstly developed from galegine, a derivative of guanidine extracted from *Galega officinalis* [15]. It is not like sulfonylureas with weight gain on usage. Most patients placed on its treatment often lose weight. Nathan[15] and Clinical Task Force of International Diabetes Federation (CTFIDF) recommend that Metformin being commenced as the first-line treatment of diabetes in a newly diagnosed case [5,15]. The transport mechanism of metformin into the cell membrane has a very low passive diffusion due to its hydrophilic chemical nature conferred by its amino groups as shown in the figure below:

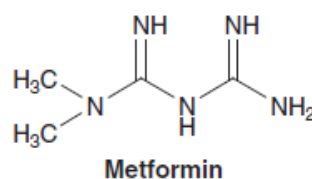


Fig.1:-Structure of Metformin

Phytochemistry of *Telfairia Occidentalis*

Telfairia occidentalis is one the readily available vegetable leaves grown in West Africa, and has a very wide range of

importance in phytomedicine. It belongs to Curcubitaceae family. It is commonly called 'Ugu or Ugwu' in Nigeria.



Fig.2:-*Telfairia occidentalis*

According to Oyekunle and Oyerele[19], *Telfairia occidentalis* is fit for human consumption as herbal medicine and in preparing soups. Nevertheless, its phytochemical screening by many scholars in the field of medicine and pharmacology has shown various biochemical functions that are unique to it. Okokon *et al.*[18] reported the leaves of *Telfairia occidentalis* as anticancer, anti-inflammatory and good antioxidant. A detailed study provided by Oboh, [7,17] also gave more phytochemical capacities of *Telfairia occidentalis* in hepato-protection and anti – HIV reverse transcription, remedying reproduction and fertility issues. It has also been apparently used in managing disease states like hypercholesterolemia, liver dysfunction and damaged immune system[26].

To limit the extent of diabetes mellitus and its complications, many synthetic therapeutic agents have been designed and developed with minimal significant success. However, the research for a better drug without adverse side effect continues, which provides a direction for the present

study. With the prevalence increase in diabetes mellitus in Nigeria, its subsequent management, its effect on human socio-economic and its challenges worldwide in term of therapeutic management, has opened windows to search for drug that is cheaper, safer and available from plants products. Iliyasu[12] and Endas[9] investigated the antidiabetic properties of *Telfairia occidentalis*, and reported that this plant possesses antidiabetic properties but their work did not specify the antidiabetic components nor its mechanism of action on most tissues like liver, kidney and their corresponding metabolites. This background initiates the current study to investigate the antidiabetic properties and phytochemical studies of fractions of ethanolic leaf extract of *Telfairia occidentalis* (*T. occidentalis*) on streptozotocin induced diabetic albino rats. The aim of this study was to evaluate the effect of ethanolic leaf extract of *Telfairia occidentalis* on diabetic induced rats with streptozotocin.

The objectives of the study include:

- To evaluate renal protection potential of ethanolic leaf extract of *Telfairia*

occidentalis in streptozotocin induced diabetic rats.

- To investigate the antidiabetic potential of ethanolic leaf extract of *Telfairia occidentalis* in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Materials

Electronic precision balance (Mettler Toledo), plastic cages, spatula, 10ml, 5ml and 1ml sterile disposable syringes and needles, oral cannula, cotton wool, Petri dish, universal bottles, normal saline, varying strengths of ethanol, citric acid, sodium citrate, distilled water, Dissecting set and ACU check (Glucometer).

Plant Material

Telfairia occidentalis (Ugu) leaves were collected from Ogbomoso, Oyo state, Nigeria in March 2018 during the early rainy season. It was then air dried at room temperature for four weeks.

Care and Management of Animals

Twenty-five male Wistar rats (*Rattus norvegicus*) weighing between 150 g and 200 g were purchased and used for this experiment. The rats were housed in the animal holding of the Department of Biochemistry, LAUTECH, Ogbomoso. Animals were housed in groups of five in plastic cages, maintained under standard dark-light cycle Food and water was available ad libitum.

Preparation of Extract of *Telfairia occidentalis*

Telfairia occidentalis leaves were first separated from the stalk, rinsed with water to remove dirt; air dried for four weeks at room temperature and ground to fine powder using an electric blender. Extraction was performed by adding 450g of ground powdered in 1.5 litres of ethanol in a sterile flask, swirled to ensure effective mixing and a stopper used to

avoid loss of volatile liquid at ambient temperature ($28 \pm 2^\circ\text{C}$). The mixture was extracted by agitation on a rotary shaker. After 48 hrs. the mixture was filtered using Whitman filter paper. The filtrate was then poured into flasks and water bath set at 40°C was used to evaporate the ethanol. The dry residue was stored in the refrigerator until ready for use.

Induction of Diabetes Mellitus

Diabetes mellitus was induced using intraperitoneal injection of 50mg/kg b.w of streptozotocin (STZ) as provided in the review of Tanko *et al.*, (2007). Streptozotocin was dissolved in 0.1 mol dm^{-3} of sodium citrate buffer at pH 4.5 (Zafar *et al.*, 2009). The control group animals were left not injected. The rats were fasted overnight before administration of STZ. 72 hours following streptozotocin injection, the rats were fasted for 8 hours and blood taken from tail vein of the rats. Rats having hyperglycaemia (that is, with blood glucose of $\geq 180 \text{ mg/dl}$ taken were considered diabetic and used for the experiment.

EXPERIMENTAL METHOD

Twenty five male Wister rats weighing between 150 and 200g were randomly assigned into five groups A, B, C, D and E of five rats each. Groups A, B, C and D had diabetes experimentally induced and Group E was the control, non-diabetic group. Group A and B received ethanolic crude extracts of *Telfairia occidentalis* leaves at 200 mg/kg (Aguiyi *et al.*, 2000) and 400 mg/kg/day respectively. Group C animals received a standard readily available antihyperglycaemic agent; Metformin at a dose of 25 mg/kg (Yanardag *et al.*, 2005). Group D animals (diabetic control) received distilled water, and Animals in group E (Control) were administered distilled water.

Table 1:-Animal groupings based on test substances administered

G r o u p s	A	B	C	D	E
Substance Administered	S T Z (50mg/kg) + Low dose Of Extract (200mg/kg)	S T Z (5 0 m g / k g) + High dose Of Extract (4 0 0 m g / k g)	S T Z (50mg/kg) + Metformin (1.43mg/kg)	S T Z (5 0 m g / k g)	Distilled Water

All drugs and reagents were administered via an oral cannula daily for a period of 8 days.

Sacrifice of Animals

At the end of the experimental period rats were observed for changes in their physical characteristics and then sacrificed by cervical dislocation and the kidneys of each of the animals dissected out through a midline abdominal incision passing through the abdominal wall musculature into the peritoneal cavity.

Preparation of Histological Samples

The kidneys excised from the test animals were fixed in 10% formalin, and then sent to University of Ilorin Teaching Hospital for histopathology analysis.

Determination of Blood Glucose

Blood glucose was measured every 48 hours (days 0, 2, 4, 6, 8) after an overnight fast by using digital glucometer that uses glucose – oxidase method provided by Trinder (1969).

Collection and Analysis of Blood Samples for Kidney Biochemistry

Blood was collected from each diabetic and non-diabetic rat on the 9th day by intracardiac puncture; rats were fasted for 8 hours before samples were collected. Samples were collected into lithium heparinised bottles; blood was allowed to clot and serum separated by centrifugation at 3500 rpm for 10 minutes using a haematocrit centrifuge. Serum creatinine

was determined by a colometric reaction (Jaffe's Method), using an autoanalyser, serum urea was measured using a colometric reaction (DAM Method).

Determination of Total Proteins Concentration

Quantitative determination of total protein concentration was done using Biuret (colourimetric) method. In an alkaline medium, proteins react with copper salts to give an intensive violet-blue complex.

Reagents

Working reagent R1 Biuret composes of:
Sodium potassium tartrate (15mmol/l)
Sodium iodide (100mmol/l)
Potassium iodide (5mmol/l)
Copper (II) sulphate (5mmol/l)
Total Protein Calibrator compose of Bovin albumin primary standard (7g/dl)

Test Procedure

1ml of protein reagent was pipetted into test tubes and 25µl of each of the standard and samples (serum and homogenized organs) was correspondingly added as shown in table 2. The solution was mixed and incubated for 5 minutes at 30-37 °C until a purple colour is established and the absorbance (A) of the sample and calibrator was read against the Blank.

Table 2:-The volume of working reagent, standard and samples pipetted into the cuvette for total protein concentration determination.

	B l a n k	S t a n d a r d	S a m p l e
W o r k i n g r e a g e n t R . 1 (m l)	1 . 0	1 . 0	1 . 0
S t a n d a r d (µ l)	–	2 0	–
S a m p l e (µ l)	–	–	2 0

The absorbance was taken at 546 nm with a cuvette of 1cm light path at ambient temperature of 37 °C. The Spectrophotometer was zero with distilled water.

Calculations;

Total Protein concentration (g/dl) =
Absorbance of Sample × Standard
concentration

Absorbance of Standard

Standard concentration = 5.5g/dl

Conversion factor, g/dl × 144.9 = µmol/l

DETERMINATION OF SERUM CREATININE CONCENTRATION

Test Principle

Creatinine reacts with picric acid in an alkaline medium to form a deep yellow

complex. The amount of complex formed is directly proportional to the level of creatinine in the sample.

Reagents

1. Sodium hydroxide (0.32mol/l)
2. Picric acid (35mmol/l)
3. Standard (2mg/dl)

Test Procedure

100µl each of standard and sample, were pipetted into test tubes representing standard and samples respectively. 1000µl of the working reagent was then pipetted into the 2 test tubes category designated as standard and sample as shown in Table 2. The resulting mixture were mixed thoroughly and absorbance of samples and standard was taken after 30 secs then again after exactly 120 secs.

Table 3:-The volume of working reagent, standard and sample are pipetted into the cuvette for serum creatinine concentration determination.

	Standard	Sample
Standard	1000 µl	-
Sample		100µl
Working Reagent	1000µl	1000 µl
Mix and read the absorbance of the standard or sample after 30 secs then again after exactly 120secs		

The absorbance was taken at 492 (490-510) nm with a cuvette of 1cm light path and ambient temperature of 37 °C.

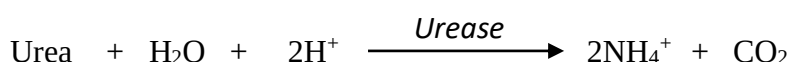
Calculation

Creatinine Concentration = $\frac{\Delta A \text{ of Sample}}{\Delta A \text{ of Standard}} \times \text{Standard concentration}$

Standard concentration = 2mg/dl

DETERMINATION OF UREA CONCENTRATION

Test Principle



Reagents

(a) Enzyme reagent R2 compose of:

2-oxoglutarate (115mmol/l)

NADH (1.44mmol/l)

(b) Buffer reagent R1 compose of:

Tris Buffer, 2 – Oxoglutarate, glutamate dehydrogenase, adenosine diphosphate, etc

(c) Standard R4 is composed of: Urea (50mg/dl)

Table 4:-The volume of working reagent, standard and sample are pipetted into the cuvette for serum urea concentration determination.

	Standard	Sample
Working Reagent	1000 µL	1000 µL
Standard	10 µL	-
Sample	-	10 µL

Test Procedure

1000µl of working reagent, were pipetted into test tubes representing blank, standard and samples respectively. 10µl each of the standard and sample were then pipetted into the 2 test tubes category designated as standard and sample as shown in table 2.13 The resulting mixture were mixed thoroughly and incubated for 15 minutes at +37°C and absorbance of samples and standard was taken against the reagent blank.

The absorbance was taken at 340nm with a cuvette of 1cm light path and ambient temperature of 37 °C.

Calculation

(T1 –T2) of Sample
Urea Conc. (mg/dL) = -----
----- x 50
(T1-T2) of standard

Statistical Analysis

Data collected were expressed as mean ± SEM (using SPSS v20.0) and subjected to both one-way and two-way analysis of variance (ANOVA) using the statistical Prism. Least significant differences (LSD) were used as a test of significance within treatments. A p-value of < 0.05 was considered statistically significant in statistical comparison.

RESULTS

Determination of Blood Glucose Concentrations in Various Treatment Groups

Table 5:-Blood glucose concentration in various treatment groups for Day 0 – Day 8

GROUPS	CONCENTRATION (mg/dl) EXPRESSED AS MEAN±SEM				
	Day 0	Day 2	Day 4	Day 6	Day 8
A (STZ + Low Dose of Extract)	504±0.01	365±1.96	325±2.16	170±0.71	114.4±4.67
B (STZ + High Dose of Extract)	501±0.00	353±1.99	300±3.37	105±1.52	90.8±0.58
C (STZ + Metformin)	503±0.00	283±7.49	272±2.52	94±3.12	97±0.86
D (STZ Only)/Untreated	499±0.00	490±2.86	466±4.14	524±3.95	410±5.75
E (Control)	102±2.00	86±1.00	99±4.27	98±2.02	99±2.84

Table 5 and Figure 3 show the mean levels of blood glucose taken every 48 hour over a 8 day period. There was a statistically significant (p<0.05) dose dependent reduction in blood glucose levels in ethanolic extract of *Telfairia occidentalis* groups with a steep descent seen at higher dose compared to the diabetic control

(STZ only). Administration of the extract caused a significant (p<0.05) reduction in blood glucose in groups A and B respectively on day 8 compared to the initial blood glucose levels. Metformin administration also resulted in a significant drop in the blood glucose level with its administration resulted in a significant

($p < 0.05$) reduction in blood glucose between days 0 and 8. The diabetic control (Streptozotocin only) group however,

maintained a significantly higher blood glucose levels compared to normal control all throughout the experimental period.

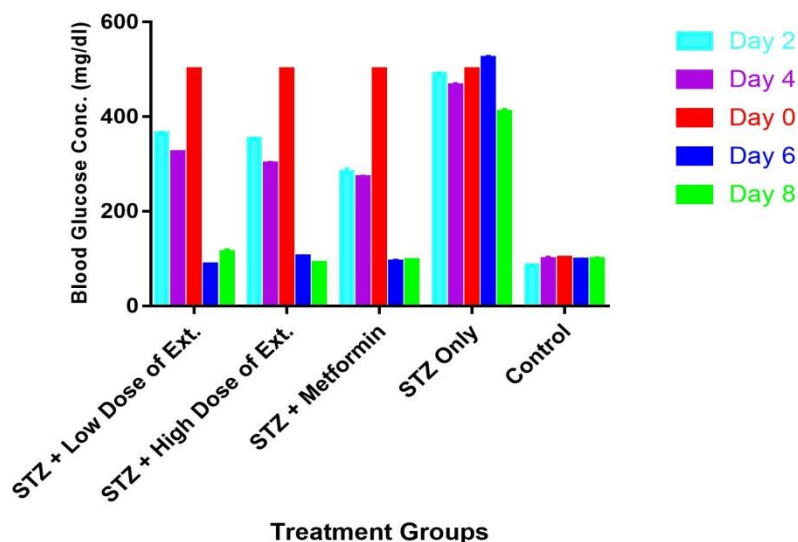


Fig.4:- Blood glucose concentration in various treatment groups over a 8 – day period

Data are expressed as Mean $\pm$ SED, $n=5$ and Level of significance was taken at $P < 0.05$ for five animals per group.

Determination of Serum Sodium (Na) Concentrations in Various Treatment Groups

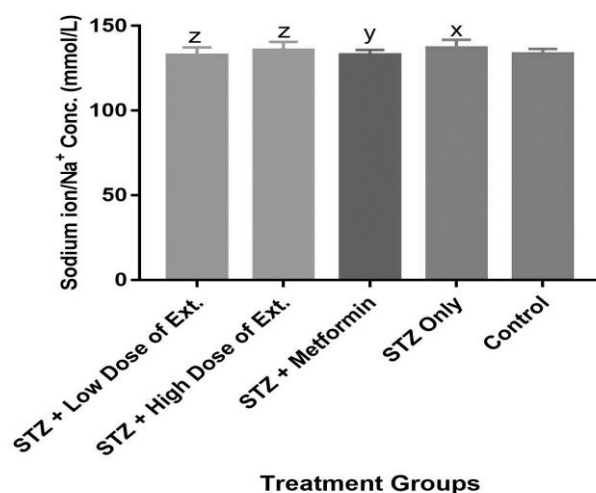


Fig.5:- Sodium concentration in various treatment groups

Data are expressed as Mean $\pm$ SEM, $n=5$ and Level of significance was taken at $P < 0.05$ for five animals per group. STZ = Streptozotocin A significant ($P < 0.05$) increase was observed in STZ – induced diabetic rats when compared to control animals. However, a significant ($P < 0.05$)

decrease was observed in STZ + Metformin group when compared to STZ – induced diabetic rats. A significant ($P < 0.05$) decrease was observed in both STZ + Low Dose of Extract and STZ + High Dose of Extract groups when compared to STZ – induced diabetic rats.

Determination of Serum Potassium (K) Concentrations in Various Treatment Groups.

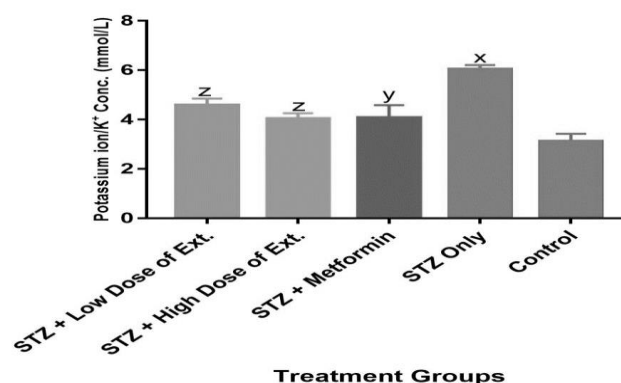


Fig.6:-Potassium concentration in various treatment groups

Data are expressed as Mean $\pm$ SEM, n=5 and Level of significance was taken at $P < 0.05$ for five animals per group. STZ = Streptozotocin A significant ($P < 0.05$) increase was observed in STZ – induced diabetic rats when compared to control animals. However, a significant ($P < 0.05$)

decrease was observed in STZ + Metformin group when compared to STZ – induced diabetic rats. A significant ($P < 0.05$) decrease was observed in both STZ + Low Dose of Extract and STZ + High Dose of Extract groups when compared to STZ – induced diabetic rats.

Determination of Serum Total Protein Concentration in Various Treatment Groups.

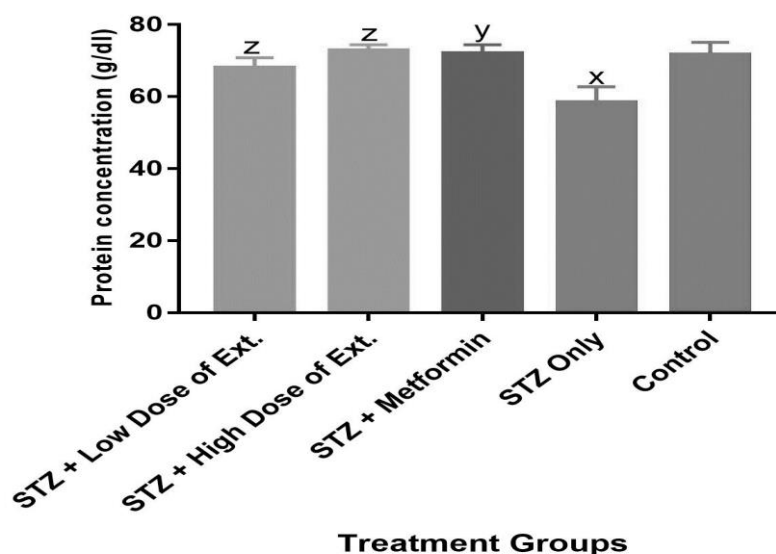


Fig.7:-Serum total protein concentration in various treatment groups

Data are expressed as Mean $\pm$ SEM, n=5 and Level of significance was taken at $P < 0.05$ for five animals per group. STZ = Streptozotocin A significant ($P < 0.05$) decrease was observed in STZ – induced diabetic rats when compared to control animals. However, a significant ($P < 0.05$) increase

was observed in STZ + Metformin group when compared to STZ – induced diabetic rats. A significant ($P < 0.05$) increase was observed in both STZ + Low Dose of Extract and STZ + High Dose of Extract groups when compared to STZ – induced diabetic rats.

Determination of Serum Albumin Concentration in Various Treatment Groups.

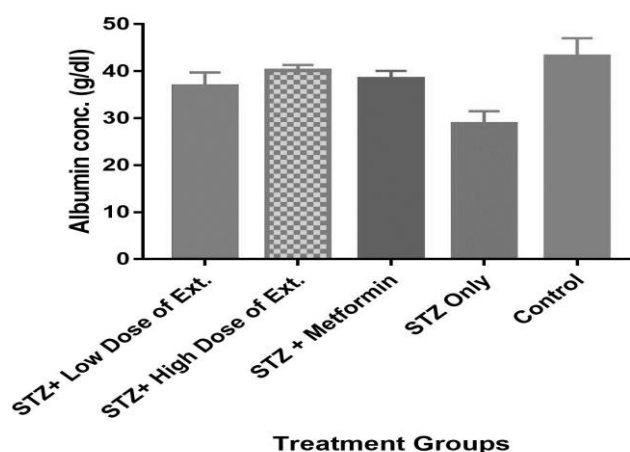


Fig.8:- Serum albumin concentration in various treatment groups

A significant ($P < 0.05$) decrease was observed in STZ – induced diabetic rats when compared to control animals. However, a significant ($P < 0.05$) increase was observed in STZ + Metformin group when compared to STZ – induced diabetic

rats. A significant ($P < 0.05$) increase was observed in both STZ + Low Dose of Extract and STZ + High Dose of Extract groups when compared to STZ – induced diabetic rats.

Determination of Serum Creatinine Concentration in Various Treatment Groups.

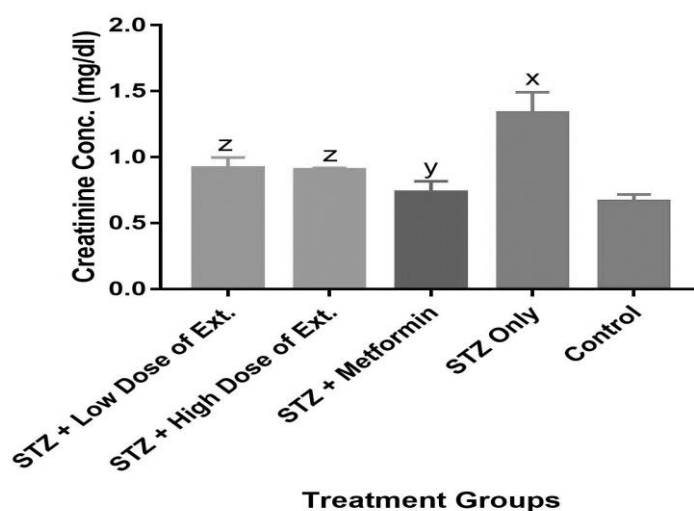


Fig.9:- Serum creatinine concentration in various treatment groups

Data are expressed as Mean $\pm$ SEM, $n=5$ and Level of significance was taken at $P < 0.05$ for five animals per group. STZ = Streptozotocin A significant ($P < 0.05$) increase was observed in STZ – induced diabetic rats when compared to control animals. However, a significant ($P < 0.05$)

decrease was observed in STZ + Metformin group when compared to STZ – induced diabetic rats. A significant ($P < 0.05$) decrease was observed in both STZ + Low Dose of Extract and STZ + High Dose of Extract groups when compared to STZ – induced diabetic rats.

Determination of Serum Urea Concentration in Various Treatment Groups.

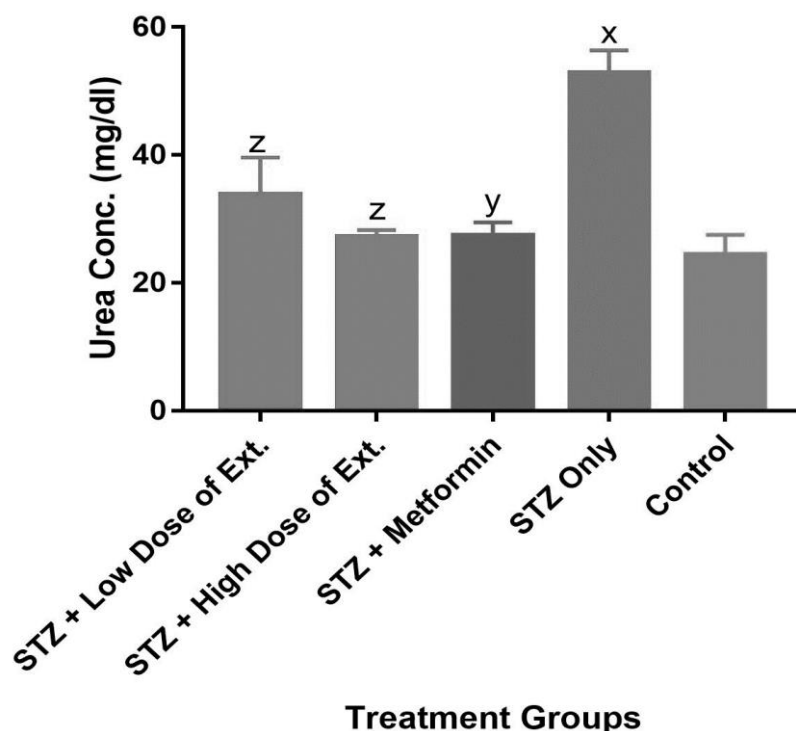
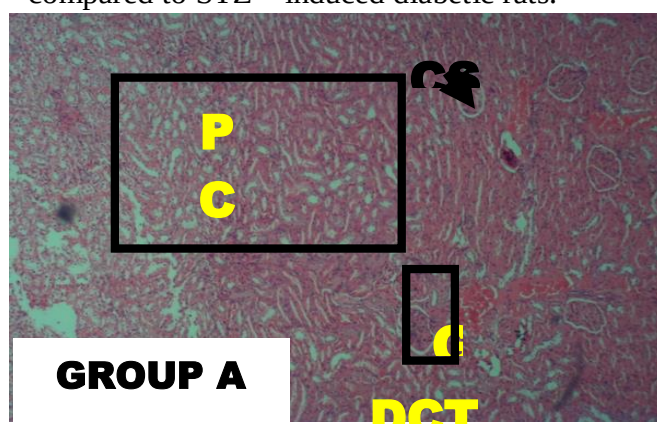
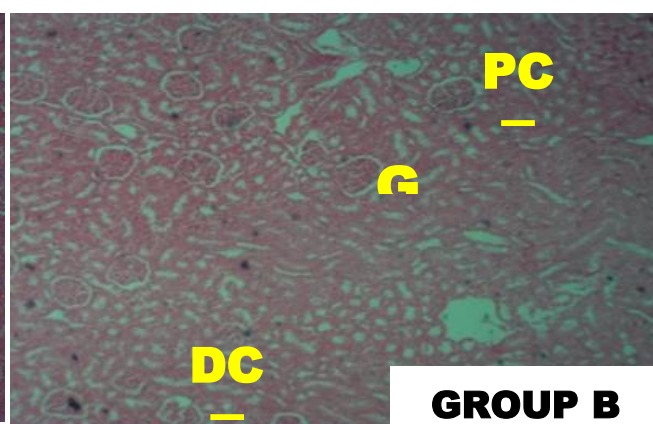


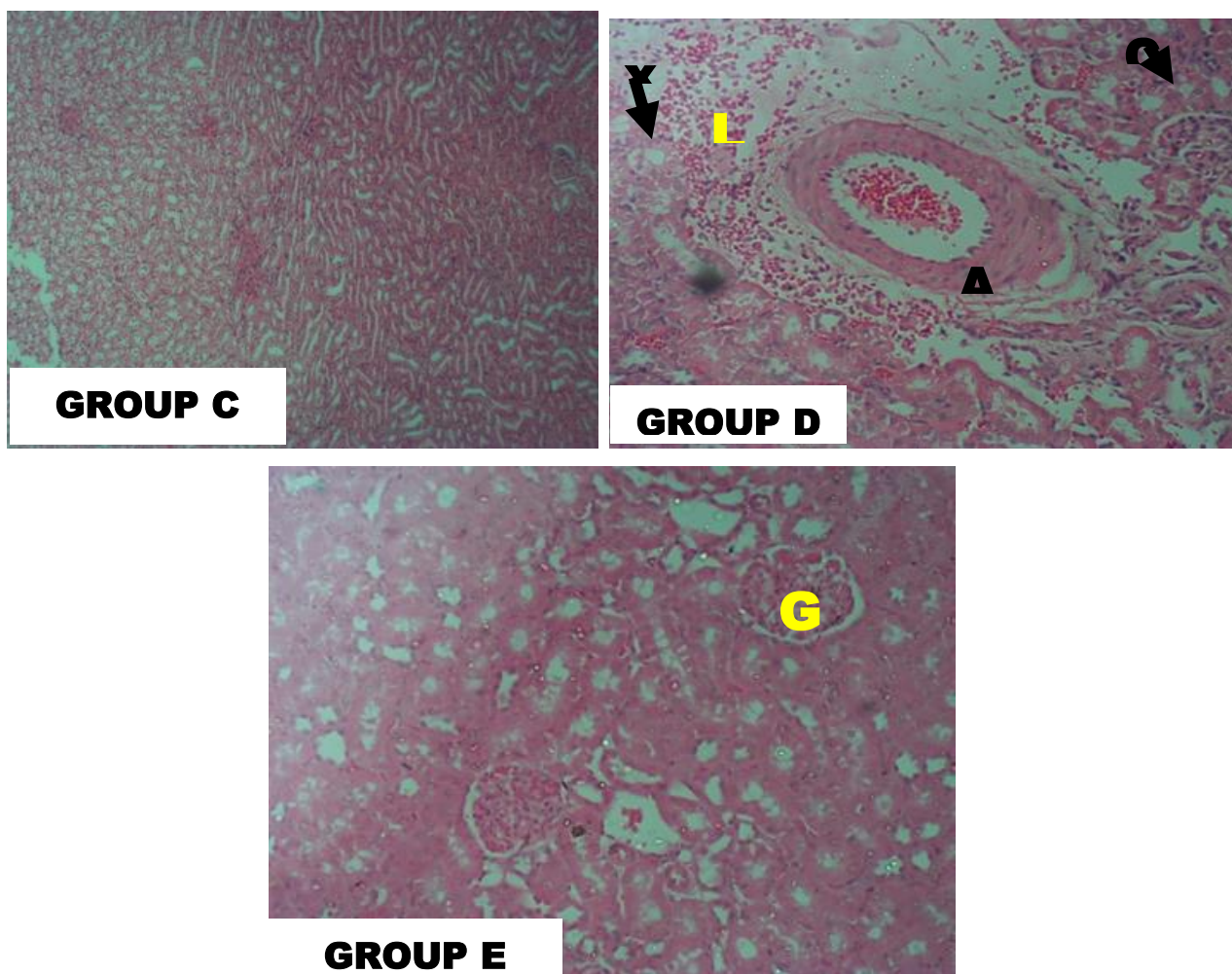
Figure 10:- Serum urea concentration in various treatment groups

Data are expressed as Mean $\pm$ SEM, n=5 and Level of significance was taken at $P < 0.05$ for five animals per group. STZ = Streptozotocin A significant ($P < 0.05$) increase was observed in STZ – induced diabetic rats when compared to control animals. However, a significant ($P < 0.05$) decrease was observed in STZ + Metformin group when compared to STZ – induced diabetic rats. A significant ($P < 0.05$) decrease was observed in both STZ + Low Dose of Extract and STZ + High Dose of Extract groups when compared to STZ – induced diabetic rats.

Result of Kidney Histopathology

Treatment Groups A, B, and C shows normal glomeruli with adequate capsular space. No significant renal cellular dissertations were observed. Treatment D (Untreated groups) shows a wide spread replacement of glomerulus by a vascular, a cellular hyaline materials (**X**), the cortical tubules (**C**) also become shrinks and atrophic. The interstitial spaces undergoes fibrosis. Heavy polymorph nuclear infiltrations (**L**) was also observed around the artery (**A**)





DISCUSSION AND CONCLUSION

Discussion

One of the leading challenges to public health globally is diabetes mellitus. Diabetes mellitus is a chronic condition caused by irregular high level of glucose in the blood. However, therapeutic option mostly adopted is the use of natural plant derivatives. The purpose of this study was also to investigate the antidiabetogenic effect of *Telfairia occidentalis* in Streptozotocin induced diabetic rats. Various results of the parameters studied showed the corresponding effect of Metformin and *Telfairia occidentalis* in diabetic induced rats.

As shown in Table 3 and Figure 1, there was a statistically significant ($p < 0.05$) dose dependent reduction in blood glucose levels in ethanolic extract of *Telfairia occidentalis* groups with a steep descent

seen at higher dose compared to the diabetic control (untreated group). Administration of the extract caused a significant ($p < 0.05$) reduction in blood glucose in groups A and B respectively on day 8 compared to the initial blood glucose levels. Metformin administration also resulted in a significant drop in the blood glucose level with its administration resulted in a significant ($p < 0.05$) reduction in blood glucose between days 0 and 8. The diabetic control (Streptozotocin only) group however, maintained a significantly higher blood glucose levels compared to normal control all throughout the experimental period, as postulated by Lenzen [14].

Abnormal sodium and potassium (hypernatremia and hyperkalaemia) have been reported to be one of the major components of high blood pressure as well

as renal failure and have been correlated with high incidence of cardiovascular diseases. Evaluation of the serum sodium and potassium levels are deployed as markers of imbalance in sodium and potassium. They were significantly elevated ($p < 0.05$) in the rats challenged with Streptozotocin (Group D) compared to the control rats (Group E). The diabetic rats treated with extract of *Telfairia occidentalis* (Group A and B) and metformin (Group C) showed a significant decrease ($p < 0.05$) in sodium and potassium levels when compared to untreated rats.

A significant decrease ($p < 0.05$) in the level of serum total protein was noted and recorded in Streptozotocin challenged diabetic rats compared to the normal control rats. The loss of protein concentration is due to protein oxidation caused by reactive oxygen species (ROS) which might have resulted to protein fragmentation and protein-protein cross-linkages as supported by Zhang *et al.* [27]. A significant reduction in albumin level is an initial stage of diabetic kidney disease. This occurs when kidneys are occupied with loads of removing too much of blood albumin consequently increasing the urine albumin with a derangement and disorder known as micro albuminuria. Furthermore, the result garnered here may intensify Streptozotocin's inhibitory action on synthesis of protein. More so, diabetic rats treated with low and high dose of extract of *Telfairia occidentalis* (Group A and B) and metformin (Group C) showed a significant increase ($p < 0.05$) in the total protein and albumin levels when compared to the untreated rats.

The STZ diabetic rats showed a significant elevation in urea and creatinine levels which indicate renal cell damage which may be due to decrease in glomerular filtration rate and may culminate into electrolyte imbalance and kidney diseases

[16]. The ethanolic extract of *Telfairia occidentalis* and metformin groups showed a reduction in the levels of these substances, indicating protection against renal injury.

Histopathology of diabetic rats showed hypertrophied and granular glomeruli with pycnotic nuclei, dilated renal tubules and numerous mononuclear cells infiltrates. The diabetic rats that received, Metformin and ethanolic extract of *T. occidentalis* showed normal glomeruli with prominent nuclei, some renal tubule dilation and increased mononuclear cell infiltration. Accumulation of glycogen in the kidney tubules as a result of the ensuing hyperglycaemia is thought to be responsible for the progression of disease in diabetic nephropathy [21].

CONCLUSION

The results of this study showed that ethanolic leaf extract of *Telfairia occidentalis* is a good antihyperglycaemic agent in lowering the blood glucose level. Hypernatremia and hyperkalaemia (diabetic ketoacidosis) caused by Streptozotocin are also lowered through the use of ethanolic leaf extract of *Telfairia occidentalis*. This may therefore suggest the effect of ethanolic leaf extract of *Telfairia occidentalis* in protecting renal damage in streptozotocin induced diabetes, and is as effective as Metformin in lowering blood glucose. Therefore, the protective mechanism of *Telfairia occidentalis* might be due to its free radical scavenging potential and antidiabetic property.

REFERENCES

1. Ahmed, S. M., Swamy, V., Gopkumar, P., & Dhanapal, R. (2005). Anti-diabetic activity of Terminalia catappa Linn. leaf extracts in alloxan-induced diabetic rats. *Iranian Journal of pharmacology and therapeutics*, 4(1), 36-0.

2. Al-Daghri, N. M., Al-Attas, O. S., Alokail, M. S., Alkharfy, K. M., Yousef, M., Sabico, S. L., & Chrousos, G. P. (2011). Diabetes mellitus type 2 and other chronic non-communicable diseases in the central region, Saudi Arabia (Riyadh cohort 2): a decade of an epidemic. *BMC medicine*, 9(1), 1-6.
3. Nakhaee, A., Bokaeian, M., Saravani, M., Farhangi, A., & Akbarzadeh, A. (2009). Attenuation of oxidative stress in streptozotocin-induced diabetic rats by Eucalyptus globulus. *Indian Journal of Clinical Biochemistry*, 24(4), 419.
4. Catanese V. M & Rona, C., (2002) Chapter 139 Secondary Forms Of Diabetes Mellitus Principles and Practice of Endocrinology and Metabolism Lippincott Williams & Wilkins Publishers.
5. IDF Clinical Guidelines Task Force. (2006). Global Guideline for Type 2 Diabetes: recommendations for standard, comprehensive, and minimal care. *Diabetic medicine*, 23(6), 579-593.
6. Dolan, M. E. (1997). Inhibition of DNA repair as a means of increasing the antitumor activity of DNA reactive agents. *Advanced drug delivery reviews*, 26(2-3), 105-118.
7. Dowluru, S. K., Sreenu. B., Narasinga. R. V. (2015) Isolation, Evaluation of Antiproliferative and Antifungal Properties of a Serine Protease Inhibitor in Some Cucurbitaceae Members: A Review, *Asian Journal of Pharmaceutical Technology & Innovation*, 03(15), 4-60. www.asianpharmtech.com
8. Eisenbarth, G.S (2002): Chapter 136 Classification, Diagnostic Tests, and Pathogenesis of Type 1 Diabetes Mellitus Principles and Practice of Endocrinology and Metabolism Lippincott Williams & Wilkins Publishers.
9. Endas, J. (2014). In vivo study on the effect ethanolic leaf extract of *Telfairia Occidentalis* and *Murraya koenigii* on alloxan monohydrate induced diabetes albino rats. Being a Research Project submitted to the Department of Biochemistry, Kaduna state university.
10. Fauci A.S.; Braunwald E.; Kasper D.L.; Hauser D.L (2008), Chapter 338. Diabetes Mellitus Harrison's Principles of Internal Medicine, 17th Edition The McGraw-Hill Companies, Inc
11. Raza, H., Prabu, S. K., John, A., & Avadhani, N. G. (2011). Impaired mitochondrial respiratory functions and oxidative stress in streptozotocin-induced diabetic rats. *International journal of molecular sciences*, 12(5), 3133-3147.
12. Iliyasu. D.(2013). In vitro study on the effect of ethanolic leaf extract of *Telfairia occidentalis* and *Murraya koenigii* on alpha amylase. Being a research project submitted to the department of biochemistry, Kaduna state university.
13. James, S. A., Omwirhiren, R. E., Joshua, I. A., & Dutse, I. (2016). Anti-diabetic Properties and Phytochemical Studies of ethanolic leaf extracts of *Murraya koenigii* and *Telfairia occidentalis* on alloxan-induced diabetic albino rats. *ornament*, 49.
14. Lenzen, S. (2008). The mechanisms of alloxan-and streptozotocin-induced diabetes. *Diabetologia*, 51(2), 216-226.
15. Nathan, D. M., Buse, J. B., Davidson, M. B., Ferrannini, E., Holman, R. R., Sherwin, R., & Zinman, B. (2009). Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of

- Diabetes. *Diabetes care*, 32(1), 193-203.
16. Nolin, T. D., & Himmelfarb, J. (2010). Mechanisms of drug-induced nephrotoxicity. In *Adverse drug reactions* (pp. 111-130). Springer, Berlin, Heidelberg.
17. Oboh, G. (2005). Hepatoprotective property of ethanolic and aqueous extracts of fluted pumpkin (*Telfairia occidentalis*) leaves against garlic-induced oxidative stress. *Journal of medicinal food*, 8(4), 560-563.
18. Okokon, J. E., Farooq, A. D., Choudhary, M. I., & Antia, B. S. (2012). Immunomodulatory, anticancer and anti-inflammatory activities of *Telfairia occidentalis* seed extract and fractions. *Int J Food Nutr Saf*, 2(2), 72-85.
19. Oyekunle, O. J., & Abosede, O. T. (2012). Growth, yield and nutritional composition of fluted pumpkin (*Telfairia occidentalis*) as affected by fertilizer types in ogbomoso, south west Nigeria. *Bulletin of Environment, Pharmacology and Life Sciences*, 1(9), 81-88.
20. Rahimi, M. (2015). Review: anti diabetic medicinal plants used for diabetes mellitus. *Bull. Environ. Pharmacol. Life Sciences*, 4(2), 163-180.
21. Ramesh, B., Viswanathan, P., & Pugalandi, K. V. (2007). Protective effect of Umbelliferone on membranous fatty acid composition in streptozotocin-induced diabetic rats. *European journal of pharmacology*, 566(1-3), 231-239.
22. Bharti, R., Ahuja, G., Sujana, G. P., & Dakappa, S. S. (2012). A review on medicinal plants having Antioxidant potential. *J Pharm Res*, 5(8), 4278-4287..
23. Krishnan, S., Cozier, Y. C., Rosenberg, L., & Palmer, J. R. (2010). Socioeconomic status and incidence of type 2 diabetes: results from the Black Women's Health Study. *American journal of epidemiology*, 171(5), 564-570.
24. Skyler J.S (2004). Determination of carboxylic acids and inorganic anions in wines by ion exchange chromatography. *J. Med. Chem.* 47:4113-4117.
25. Sy, G. Y., Cissé, A., Nongonierma, R. B., Sarr, M., Mbodj, N. A., & Faye, B. (2005). Hypoglycaemic and antidiabetic activity of aceton extract of *Vernonia colorata* leaves in normoglycaemic and alloxan-induced diabetic rats. *Journal of ethnopharmacology*, 98(1-2), 171-175.
26. Usman, J. G., Sodipo, O. A., Kwaghe, A., & Sandabe, U. K. (2015). Uses of *Cucumis metuliferus*: A Review. *Cancer Biol*, 5, 24.
27. Zhang, W., Xiao, S., & Ahn, D. U. (2013). Protein oxidation: basic principles and implications for meat quality. *Critical reviews in food science and nutrition*, 53(11), 1191-1201.