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Normoglycaemic Action of *Ficus exasperata* (Vahl.) Leaf-based Diet on Fructose and Streptozotocin-induced Diabetic Rats

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Abstract: Type 2 diabetes mellitus has continued to increase globally. This has imposed considerable burdens on patients, families and society. The aim of this study was to evaluate the normoglycaemic potential of *Ficus exasperata* leaf-based diet (FELD) on fructose and streptozotocin-induced diabetic rats. Forty-eight Wistar rats were randomly selected into 8 groups designated A - H and treated as follows: A (non-diabetic), B (diabetic) and C (diabetic and metformin [12.14 mg/kg b.wt.]), while D – H were diabetic rats administered FELD containing the plant to the tune of 100 (10 %), 200 (20 %), 300 (30 %), 400 (40 %) and 500 g/kg (50 %) respectively. Fasting blood glucose (FBG), hepatic glucose, glycogen and glycosylated haemoglobin concentrations, homeostatic model assessment for insulin resistance and β score (HOMA IR and β score) and hexokinase activity were analysed. Secondary metabolite constituent and proximate analyses revealed the presence of saponins, tannins, phenolics, magnesium, zinc, ash, carbohydrate, protein, fiber, fat and moisture. Consumption of 30, 40 and 50 % FELD for 16 days reduced FBG, hepatic glucose, glycosylated haemoglobin concentrations and HOMA IR significantly ($p<0.05$) in groups D to H when compared with diabetic animals, whereas their hepatic glycogen concentration, HOMA β score and hexokinase activity increased significantly ($p<0.05$). In conclusion, 30 % FELD elicited normoglycaemic effect and as such *Ficus exasperate* leaf can be incorporated into feed for the management of hyperglyeamia at this dose.

KEYWORDS: *Ficus exasperata*; Normoglycemia; Type 2 diabetes mellitus; Streptozotocin

1.0 Introduction

Normoglycemia is a state where plasma glucose values throughout a 24-hours period is approximately 90 mg/dl, with a maximal concentration usually not exceeding 165 mg/dl (after meal ingestion) and remaining above 55 mg/dl (after exercise) or a moderate fast (6 hours) (Shrayyef and Gerich, 2010). This narrow range defining normoglycemia is maintained through an intricate regulatory and counter-regulatory neuro-hormonal system: A decrease in plasma glucose as little as 20 mg/dl (from 90 to 70 mg/dl) will suppress the release of insulin and will decrease glucose uptake in certain areas of the brain (e.g., hypothalamus where glucose sensors are located); this will activate the sympathetic nervous system and trigger the release of counter-regulatory hormones (glucagon, catecholamines, cortisol, and growth hormone) (Gerich, 1988). All these changes will increase glucose release into plasma and decrease its removal so as to restore normoglycemia. On the other hand, a 10 mg/dl increment in plasma glucose will stimulate insulin release and suppress glucagon secretion to prevent further increments and restore normoglycemia. Glucose in plasma either comes from dietary sources, breakdown of glycogen in liver (glycogenolysis) or the formation of glucose in liver and kidney from other carbons compounds (precursors) such as lactate, pyruvate, amino acids, and glycerol (gluconeogenesis).

Hyperglycemia is a condition in which an excessive amount of glucose circulates in the blood. A subject with a consistent range between approximately 5.6 and 7 mmol/l (100 – 126 mg/dl) is considered hyperglycemic, while above 7 mmol/l (126 mg/dl) is generally held to have diabetes (ADA, 2013). Hyperglycemia is caused by increased hepatic production of

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glucose, combined with diminished peripheral use, a condition that characterizes diabetes mellitus.

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (ADA, 2013). Diabetes mellitus remains one of the leading cause of death worldwide (WHO, 2013). About 347 million people globally were estimated to have diabetes (WHO, 2013). In Sub-Saharan Africa, it was estimated that 8 % of the population above 25 years had diabetes (WHO, 2013) while more than 1.56 million cases of diabetes were reported in Nigeria in 2015 (IDF, 2015). Prolonged exposure to elevated plasma glucose levels (exceeding 7 mmol/l (126 mg/dl) leads to toxic effects in a variety of cell types (glucose toxicity). In humans, a strong relationship between chronic hyperglycemia and impaired function of vascular endothelium have been documented (Coutinho *et al.,* 1999) with particular damage observed in retinal capillary endothelial cells and mesangial cells in the renal glomerulus (Coutinho *et al.,* 2003). In addition, injury to the microvasculature of the small vessels that supply the nerves contributes to neuropathy, which develops in 60 to 70% of all patients with DM (WHO, 2013).

These complications are not only very expensive to manage but possess substantial economic burden on the healthcare delivery system of the country. In addition, some of the indirect costs include disability, loss of productivity caused by absenteeism and premature death. In the light of the impact of complications associated with DM, there is a need for therapies that will maintain plasma glucose at a normal level to prevent or delay the effects of micro- and macrovascular damage.

Orthodox therapies for the management of diabetes include administration of exogenous insulin and oral hypoglycaemic agents such as biguanides and sulfonylureas. These agents often come with some side effects like hypoglycemia, weight gain, headache and dizziness (Pareek *et al.,* 2009). These limitations have necessitated the search for new anti-diabetic drugs.

In traditional African societies, phytotherapy is highly valued and widely utilized for the management of various human ailments, including diabetes mellitus and one of such plant is *Ficus exasperata* leaf. It is commonly known as sand paper tree ("Ewe ipin" in Yoruba) and is widely spread in West Africa. The Yoruba-speaking people of western Nigeria often employ the decoctions and infusions of *F. exasperata* leaf traditionally for the management, control and/or treatment of diabetes mellitus and hypertension. Several reports indicated that the extracts of *Ficus exasperata* leaf have anti-diabetic potentials (Sonibare *et al.,* 2006; Stephen *et al.,* 2011; Kazeem *et al.,* 2013; Yakubu *et al.,* 2014). However, the diet based therapy would improve compliance and adherence to drug use by patients through providing a psychological feeling in the users that they are feeding on food rather than drug (Sonibare *et al.,* 2006; Mann *et al.,* 2009; Ajayi *et al.,* 2012; Davies *et al.,* 2013). The aim of this study therefore, was to evaluate the normoglycemic potential of FELD on fructose and streptozotocin-induced diabetic rats.

2.0 Materials and Methods

2.1 Materials

2.1.1 Plant material

Ficus exasperata leaf was collected within Ilorin community. It was authenticated (voucher number UILH/001/883) at the herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

2.1.2 Experimental animals

Male Wistar rats (100 – 150 g) of norvegicus strain were obtained from the small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were housed in well ventilated cages and allowed to acclimatize to animal house conditions (temperature: 28 – 31ºC; photoperiod: 12 hour natural light and 12 hour dark; humidity: 50–55%) for 7 days. They were fed on normal rat pellet and tap water.

2.1.3 Chemical and assay kits

Streptozotocin was a product of Sigma-Aldrich, St. Louis, MO, USA. Accu check active glucometer and strips were products of Roche Diagnostic, Mannheim, Germany. Metformin was a product of Austell Laboratories Pvt. Ltd., Johannesburg, South Africa, while fructose was a product of Nature's Choice™ Wholefood Specialists, Meyerton, South Africa. Assay kits for glycosylated haemoglobin and serum insulin were products of Centronic GmbH Am Kleinfeld, Wartenberg, Germany. All other chemicals were of analytical grades and prepared in all glass apparatus using distilled water.

2.1.4 Ethical clearance

Ethical clearance for this study was obtained from the University of Ilorin Animal Ethical Committee and approval was given with protocol identification code UERC/LSC 067.

2.2 Methods

2.2.1 Plant preparation

Fresh leaf of *F. exasperata* was collected; air-dried to a constant weight, pulverized using an electronic blender, stored in air tight container and kept in the refrigerator prior to analyses.

2.2.2 Feed materials and processing

Corn starch was prepared using yellow corn obtained from a local market in Ilorin, Kwara State, Nigeria. It was rinsed and soaked for 48 hours, ground and sieved to remove shaft. The filtrate was oven-dried at 40^{o}C to constant weight. Maize husk was also purchased from a local market in Ilorin, sun-dried and pulverized using a commercial grinder. Soy bean, sucrose and soy bean oil were all purchased from the market as well. Soy bean grain was soaked in water for 6 hours and the seed coats were removed. Thereafter, it was sun-dried and pulverised. Vitamin and mineral mix, D-methionine and L-lysine were products of Rofat Feed Nigeria Limited, Ilorin, Nigeria.

Corn starch, maize husk (cellulose source), sucrose, ground soybean, soybean oil, vitamin/mineral mix, D- methionine, L-lysine were thoroughly mixed with pulverized *F. exasperata* leaf at different concentrations (i.e. 100, 200, 300, 400 and 500 g/kg). Water was added until the mixure became a paste. The paste was then grated on a wire mesh to form pellet and oven-dried at 40^{o}C to a constant weight.

2.2.3 Qualitative secondary metabolites screening of F. exasperata leaf

Qualitative secondary metabolites of *F. exasperata* leaf was done using the methods of Sofowora (1993) as described:

2.2.3.1 Test for saponins

The pulverized *F. exasperata* leaf (1 g) was boiled with 10 ml of distilled water in a water bath for 10 minutes. The mixture was filtered while hot and allowed to cool. The filtrate (2.5 ml) was diluted to 10 ml with distilled water and shaken vigorously for 2 minutes, formation of froth which was stable for some minutes indicated the presence of saponins.

2.2.3.2 Test for terpenoids

Pulverized *F. exasperata* leaf (2 g) was dissolved in 20 ml of distilled water and 5 ml of the filtrate was then mixed with 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was added to form a layer and a reddish-brown precipitate colouration at the interface formed indicated the presence of terpenoids.

2.2.3.3 Test for flavonoids

One gram of pulverized *F. exasperata* leaf was boiled in 20 ml of distilled water and then filtered. Five (5) ml of dilute ammonia solution was added to 6 ml of the filtrate, followed by the addition of 2 ml of concentrated H_2SO_4. A yellow coloration was indicative of the presence of flavonoids.

2.2.3.4 Test for tannins

One gram of pulverized *F. exasperata* leaf was boiled with 20 ml of distilled water for five minutes in a water bath and filtered while hot. The cooled filtrate (1 ml) was diluted to 5 ml with distilled water and a few drops of 10% ferric chloride was added and observed. Formation of precipitate and blue-black colouration confirmed the presence of tannins.

2.2.3.5 Test for phenolics

Two grams of pulverized *F. exasperata* leaf was percolated in distilled water for 10 minutes and filtered. Two drops of 5 % $FeCl_3$ was added to 1 ml of the filtrate. A greenish precipitate was taken as indication for the presence of phenolics.

2.2.3.6 Test for steroids

One gram of pulverized *F. exasperata* leaf was dissolved in 2 ml of chloroform. Concentrated H_2SO_4 (0.2 ml) was carefully added to form a layer. A reddish-brown colour at the interface between the layers was formed which indicated the presence of steroids.

2.2.3.7 Test for reducing sugars

Ten millilitres of distilled water was added to 1 g of pulverized *F. exasperata* leaf and boiled for 5 minutes. The mixture was filtered while hot and then cooled. Five millilitres of mixture of equal volumes of Fehling's solution (A and B) was added to 2 ml of the filtrate in a test tube and the resultant mixture was boiled for 2 minutes. Appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugars.

2.2.4 Quantitative secondary metabolites analyses of Ficus exasperata leaf

The total phenolic contents of *F. exasperata* leaf were estimated using the Folin Ciocalteau reagent as described by Singleton and Rossi (1965). Aluminum chloride colorimetric method as described by Chang *et al*. (2002) was used for the determination of total flavonoids. Total saponins content in *F. exasperata* leaf were quantified spectrophotometrically following the method of Hiai *et al.* (1976). The concentration of tannins was determined by Folin-Ciocalteu method (Govindappa *et al*., 2011). Terpenoids, steroids, reducing sugars, cyanogenic glycosides and phytate were quantified using the method described by Malik *et al*. (2017), Trease an Evans (1989), Miller (1959), Amadi *et al*. (2004) and Latta and Eskin (1980) respectively.

2.2.5 Proximate analysis of Ficus exasperata leaf

Moisture content and crude protein were determined using the methods described by Pearson (1981)**.** Ether extract, crude fibre, ash and carbohydrate content were determined using the methods of Association of Official Analytical Chemists (AOAC, 2005).

2.2.6 Determination of mineral composition of Ficus exasperata leaf

The mineral constituents were quantified using X-Ray fluorescence (XRF) transmission emission technique with XR-100CR Si detector, PX2CR power supply and shaping amplifier. The pulverised sample was pelletized and then irradiated with X-Ray for 1000 seconds to obtain the characteristics spectra. Each spectrum was made up of peaks which were characteristics of certain elements contained in the sample. The spectrum was checked on the computer system and then interpreted for quantitative determination of minerals by direct comparison of count rates.

2.2.7 Diabetes induction and determination of blood glucose

All experimental rats apart from those in the non-diabetic control group were given 10% fructose solution *ad libitum* for 2 weeks. Rats in the normal control group were given distilled water. At the end of the 2 weeks administration of fructose solution, rats were fasted overnight and each of the fructose-fed rats were injected (intraperitoneally) with a freshly prepared 40 mg/kg b.w. streptozotocin (STZ) (dissolved in

0.1 M citrate buffer, pH 4.5) (Wilson and Islam, 2012). Seven (7 days) after STZ injection, fasting blood glucose concentration of the rats were taken, rats showing fasting blood glucose concentration above 200 mg/dl were considered hyperglyceamic.

2.2.8 Animal grouping and feed administration

Forty-eight male albino rats were randomly assigned into 8 groups of 6 animals each:

C	Normal control (non-diabetic rats fed on formulated diet without *F. exasperata* leaf)
D	Negative control (diabetic rats fed on formulated diet without *F. exasperata* leaf)
D+Met	Diabetic rats fed on formulated diet without *F. exasperata* leaf and treated with 12.14 mg/kg body weight (b.wt.) of metformin
D+10%	Diabetic rats fed on diet containing 100 g/kg of *F. exasperata* leaf
D+20%	Diabetic rats fed on diet containing 200 g/kg of *F. exasperata* leaf
D+30%	Diabetic rats fed on diet containing 300 g/kg of *F. exasperata* leaf
D+40%	Diabetic rats fed on diet containing 400 g/kg of *F. exasperata* leaf
D+50%	Diabetic rats fed on diet containing 500 g/kg of *F. exasperata* leaf

Formulated diet was administered to experimental rats *ad libitum* for a 16 days.

2.2.9 Animal sacrifice and tissue collection

Rats were sacrificed 24 hours after the last day of treatment. They were anaesthetized with diethyl ether and sacrificed by simply incising the jugular veins with scalpel. Blood samples were collected into plain sample bottles for biochemical analysis. After sacrifice, the rats were dissected in order to excise the liver. The excised organ was then rinsed in ice cold 0.25 M sucrose solution, cleansed with cotton wool to remove blood stains, weighed and immediately stored in ice cold 0.25 M sucrose solution. A weighed portion of the liver was cut with a clean scalpel and homogenized in ice-cold 0.25 M sucrose solution ($1:5^{w}/_{v}$). Serum was collected after allowing the blood sample to clot at room temperature. The supernatant (serum) was then collected using a Pasteur's pipette.

2.2.10 Determination of fasting blood glucose

Fasting blood glucose (FBG) concentration of all experimental groups was determined using a glucose oxidase-based glucometer. Blood was withdrawn from the caudal vein of the rat tail and dropped on the center of the green field on the strip. The test strip was gently inserted into the glucometer and the FBG concentration in mg/dl was displayed on the glucometer. After induction of diabetes has been confirmed, FBG concentration was determined every 3 days throughout the experimental period.

2.2.11 Determination of serum insulin concentration

Serum insulin concentration was determined by using an ultrasensitive insulin ELISA kit and concentration read using a multi plate ELISA reader (Biorad- 680, BIORAD Ltd., Japan) on day 17 (twenty-four hours after the last day of treatment). Fifty microliter (50 µl) of the calibrator (of different concentration) and sample (serum) were pipetted into the appropriate wells. Then, 100 µl of insulin reagent was added to each well. The microplate was then covered with a plastic wrap and swirled gently for 30 seconds for proper mixing. The mixture was incubated for 120 minutes at room temperature. Thereafter the contents of the microplate were discarded by decantation. This was followed by the addition of 350 µl of wash buffer afterwhich 100 µl of insulin working substrate solution was added to all wells and incubated at room temperature for 15 minutes. Fifty microliters of insulin stop solution was then added to each well and mixed gently for 20

seconds. The absorbance of each sample and calibrator was read at 450 nm in a microplate reader. A calibration curve for insulin (i.e. the graph of absorbance of the calibrator against their concentrations) was used in determining the concentration of insulin in the serum samples by extrapolating the absorbance obtained on the graph and multiplying by the dilution factor.

2.2.12 Assay of hepatic hexokinase activity

The method described by Akinyosoye *et al.* (1987) was used for this assay. Two microliters (2 µl) of 0.2 M tris buffer was pipetted into sample and blank test tubes, then 0.2 ml of 0.09 g/ml of glucose was added to each test tube. Also, 0.1 ml of 10 Mm ATP and 0.3 ml of 10 mM $MgCl_2$ were added to each of the test tubes. Liver homogenate was centrifuged at 3000 x *g* for 5 minutes, 0.1 ml of liver supernatant was added to the sample test tubes after which the mixture was incubated for 15 minutes at 30^{o}C before adding 0.5 ml of 5% TCA to stop the reaction. In the blank test tubes, 0.1 ml of liver supernatant was added after the addition of 5% TCA. The initial absorbance was read immediately the cuvette was inserted into a spectrophotometer at 340 nm and at exactly 1 minute after another absorbance was read.

Calculation:

Specific activity (µmol mg^{-1} min^{-1}) =

$$\frac{\Delta OD/minute \times DF}{0.1 \times TP}$$

Where: ΔOD/minute = absorbance of the sample at 1 minute – Initial absorbance of the sample, DF = dilution factor, 0.1 = volume of diluted tissue supernatant used; TP = Total Protein Concentration (mg/ml).

2.1.13 Determination of homeostatic model assessment for insulin resistance and pancreatic beta scores

Homeostatic model assessment for insulin resistance (HOMA-IR) and homeostatic model assessment for beta scores (HOMA- β scores) were determined by using the following expression:

HOMA-IR =

$$\left[Insulin\left(\frac{\mu U}{l}\right) \times Bloodglucose\left(\frac{mmol}{l}\right)\right] \div 22.5$$

HOMA-β =

$$[20 \times insulin\,(\mu U/l)] \div [Blood\ glucose(mmol/l) - 3.5]$$

The calculation was based on the insulin concentration and fasting blood glucose. Conversion factor: Insulin (1U/l = 7.174 pmol/l) and blood glucose (1 mmol/l = 18 mg/dl) (Mattew *et al*., 1985).

2.2.14 Determination of glycosylated haemoglobin (HbA1C) concentration

Glycosylated haemoglobin (HbA1c) was determined by the method of Nayak and Pattabiraman (1981). The saline washed erythrocytes (0.5 ml) were lysed with 5.5 ml of water, mixed and incubated at 37°C for 15 minutes. The contents were centrifuged and the supernatant discarded. Thereafter, 0.5 ml of saline was added, mixed and processed for estimation. A known volume (4 ml) of oxalate hydrochloric solution was added to 0.02 ml of aliquot and mixed. The contents were heated at 100 °C for 4 hours, cooled and precipitated with 2 ml of 40% trichloroacetic acid. The mixture was centrifuged and to 0.5 ml of supernatant, 0.05 ml of 80% phenol and 3.0 ml of concentrated H_2SO_4 were added. The absorbance was read at 480 nm after 30 minutes and the concentration of glycogylated haemoglobin was read from the calibration curve.

2.2.15 Determination of hepatic glycogen and glucose concentration

The concentrations of hepatic glycogen and hepatic glucose were determined according to the methods described by Passoneau and Lauderdale (1974) and Trinder (1969) respectively.

2.2.16 Statistical analysis

All data were expressed as the mean of six determinations ± standard error of mean (S.E.M). Statistical evaluation of data was performed by SPSS version 16.0 using one way analysis of variance. *Post hoc* comparison was done using Duncan's multiple range test. Values were considered statistically significant at $p < 0.05$ (confidence level = 95%).

3.0 Results

The *F. exasperata* leaf contained high concentrations of saponins (68.80 mg/g) and flavonoids (55.20 mg/g) (Table 1). The concentration of cyanogenic glycosides was above regulatory threshold (309.85 mg/100 g) but was drastically reduced (5.56 mg/100 g) after oven treatment (Table 2). Mineral revealed that potassium was the highest element (1.20 mg/100 g) in terms of concentration followed closely by sodium (0.67 mg/100 g) and other elements such as zinc, iron, manganese and chromium (Table 3). Nutrient composition of *F. exasperata* leaf showed that it contained a significant amount of the basic nutrients such as ash (27.10%), fibre (10.76%) and protein (17.91%) contents (Table 4).

The maintainance of animals on fructose and STZ showed that the fasting blood glucose level ranged from 300- 400 mg/dl. Furthermore, all the treatment groups had significantly ($p<0.05$) reduced fasting blood glucose levels to about 100 mg/dl with rats fed 30% *F. exasperata* leaf-based diet comparing favourably with the control animals.

Glycosylated haemoglobin of negative control animals (diabetic) was significantly ($p<0.05$) higher (72.71 mmol/mol Hb) than the normal control group (49.67 mmol/mol Hb). There was no significant difference ($p>0.05$) between the treatment groups and normal control at the end of the experiment. The serum insulin level was significantly ($p>0.05$) reduced for the negative control (D) when compared with the normal control (Table 5).

HOMA IR for the negative control was significantly higher ($p<0.05$) compared to positive control, while HOMA β-Scores was reduced significantly ($p<0.05$) for the negative control before the commencement of treatment (Table 6).

Fasting blood glucose reduced significantly ($p<0.05$) in the various levels of inclusion of the *F. exasperata* leaf compared to the diabetic animals and compared favourably with the positive control animals (Figure 1). Furthermore, hepatic glucose concentration reduced significantly in animals maintained on the leaf-based diet (Figure 2) whereas the hepatic glycogen content increased significantly ($p<0.05$) in the animals maintained on FELD when compared with the diabetic animals only (Figure 3).

4.0 Discussion

In this study, the result of the qualitative and quantitative secondary metabolites showed that leaf of *F. exasperata* contained saponins, flavonoids, tannins, terpenoids, phenolics and reducing sugars. Alklaoids, anthraquinones and phlobatannis on the other hand were not detected. This observation is contrary to some findings from previous studies that reported the presence of alkaloids among the secondary metabolites in *F. exasperata* (Odutuga *et al*., 2014). These secondary metabolites might be responsible for the normoglycemic effect elicited by FELD in the present study. Previous studies suggested that these secondary metabolites confer medicinal properties on plants (Prabhakar and Dobie, 2008; Chikezie *et al*., 2015). For example, tannin supplement from *Ficus racemosa* was shown to ameliorate hyperglycemia in streptozotocin-induced hypercholesterolemia associated diabetic rats (Velayutham *et al*., 2012). Saponins isolated from *Gymnema sylvestre* has also been shown to be agents of glycemic control in animal models (Sugihara *et al*., 2000; Yu *et al*., 2003). Flavonoids as well as phenolics have been reported to possess antihyperglycemic effect (Manickam *et al.,* 1997; Lu *et al.,* 2009). The significant decrease in the cyanogenic glycosides concentration after oven treatment suggests that pre-treating *F. exasperata* leaf before consuming might reduce the chances of poisoning from cyanide hydride. Anti-nutrients are found at some level in almost all foods. However, traditional methods of food

Table 1: Secondary Metabolite Constituents of *Ficus exasperata* Leaf Extract

Secondary metabolites	Quantity (mg/g)
Saponins	68.80 ± 4.20
Flavonoids	55.20 ± 4.62
Tannins	14.12 ± 2.45
Terpenoids	28.90 ± 4.55
Phenolic	45.61 ± 2.24
Reducing sugar	28.80 ± 4.20
Steroids	4.20 ± 0.42
Alkaloids	Not detected
Anthraquinones	Not detected
Phlobatanins	Not detected
Cardiac glycosides	Not detected

Values are means of 3 determinations ± SD

Table 2: Antinutrient Composition of *Ficus exasperata* Leaf Extract

Antinutrients	Quantity (mg/100g)	
Phytate	0.01×10^{-2} ± 0.00	
Cyanogenic glycosides	309.85 ± 8.50	*5.56 ± 0.42

Values are means of 3 determinations ± SD; *Value after oven treatment at 11 ^{o}C for 6 minutes

Table 3: Mineral Composition of *F. exasperata vahl* Leaf Extract

Minerals	Quantity (mg/100 g)
Zinc (Zn)	0.10 ± 0.09
Iron (Fe)	0.27 ± 0.08
Potassium (K)	1.20 ± 0.08
Magnesium (Mg)	0.08 ± 0.04
Sodium (Na)	0.67 ± 0.07
Copper (Cu)	0.22 ± 0.01
Manganese (Mn)	0.05 ± 0.01
Cobalt (Co)	0.11 ± 0.06
Chromium (Cr)	0.03 ± 0.01

Values are means of 3 determinations ± SD

Table 4: Nutrients Composition of *Ficus exasperata* Leaf

Nutrients	Concentration (%)
Carbohydrate	25.38 ± 0.20
Protein	17.91 ± 0.08
Fat	9.29 ± 0.06
Ash	27.10 ± 0.24
Fibre	10.76 ± 0.06
Moisture	9.56 ± 0.07

Values are means of 3 determinations ± SD

Table 5: Hexokinase Activity, Glycosylated Haemoglobin and Serum Insulin Concentrations of Fructose and Streptozotocin-induced Diabetic Rats Treated with *F. exasperata* Leaf-based Diet

Group	Hexokinase activity (µmol mg^{-1} min^{-1})	Glycosylated haemoglobin(mmol/mol Hb)	Plasma Insulin (µU/ml)
Control	2.43 ± 0.15^{a}	49.67 ± 1.53^{a}	20.33 ± 1.40^{a}
Diabetic	0.57 ± 0.01^{b}	72.71 ± 0.59^{b}	18.33 ± 0.88^{a}
Diabetic + Met	2.35 ± 0.19^{a}	52.11 ± 1.70^{a}	20.33 ± 0.33^{a}
Diabetic + 10%	2.55 ± 0.25^{a}	50.69 ± 0.33^{a}	20.67 ± 1.20^{a}
Diabetic + 20%	2.64 ± 0.01^{a}	51.07 ± 2.82^{a}	19.00 ± 0.57^{a}
Diabetic + 30%	2.75 ± 0.01^{a}	50.22 ± 2.42^{a}	18.67 ± 0.88^{a}
Diabetic + 40%	2.56 ± 0.02^{a}	47.68 ± 0.72^{a}	19.33 ± 0.57^{a}
Diabetic + 50%	2.69 ± 0.04^{a}	47.74 ± 2.95^{a}	19.00 ± 0.57^{a}

Values are means of 6 determinations ± S.E.M. and those with different superscripts along a column are statistically different ($p < 0.05$); 10, 20, 30, 40 and 50% are inclusion levels of *F. exasperata* leaf

Table 6: Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and Beta Scores (HOMA β-scores) in Type 2 Diabetic Rats

	Day 1		Day 16	
Group	**HOMA IR**	**HOMAβ-Score**	**HOMA-IR**	**HOMA β-Scores**
Control	9.77 ± 0.24^{a}	247.40 ± 0.43^{a}	6.74 ± 0.64^{a}	262.44 ± 1.58^{a}
Diabetic	26.75 ± 1.82^{b}	46.20 ± 0.21^{b}	35.35 ± 3.42^{b}	33.80 ± 1.37^{b}
D+Met	24.78 ± 1.38^{b}	47.19 ± 1.27^{b}	8.89 ± 0.48^{a}	221.62 ± 0.67^{a}
D+10%	25.68 ± 0.77^{b}	44.93 ± 0.42^{b}	8.47 ± 0.38^{a}	256.00 ± 2.10^{a}
D+20%	26.21 ± 2.18^{b}	44.24 ± 0.72^{b}	8.02 ± 0.26^{a}	263.00 ± 1.17^{a}
D+30%	23.52 ± 1.53^{b}	45.91 ± 0.28^{b}	7.79 ± 0.64^{a}	248.47 ± 0.63^{a}
D+40%	26.17 ± 1.02^{b}	42.25 ± 0.13^{b}	8.88 ± 0.60^{a}	250.39 ± 2.23^{a}
D+50%	26.64 ± 1.87^{b}	44.38 ± 0.43^{b}	8.71 ± 0.76^{a}	247.26 ± 2.54^{a}

Values are expressed as mean of 6 determinations ± S.E.M. and those with different superscripts along a column are statistically different ($p < 0.05$); D = Diabetic; Met = Metformin; 10, 20, 30, 40 and 50% are inclusion levels of *F. exasperata* leaf

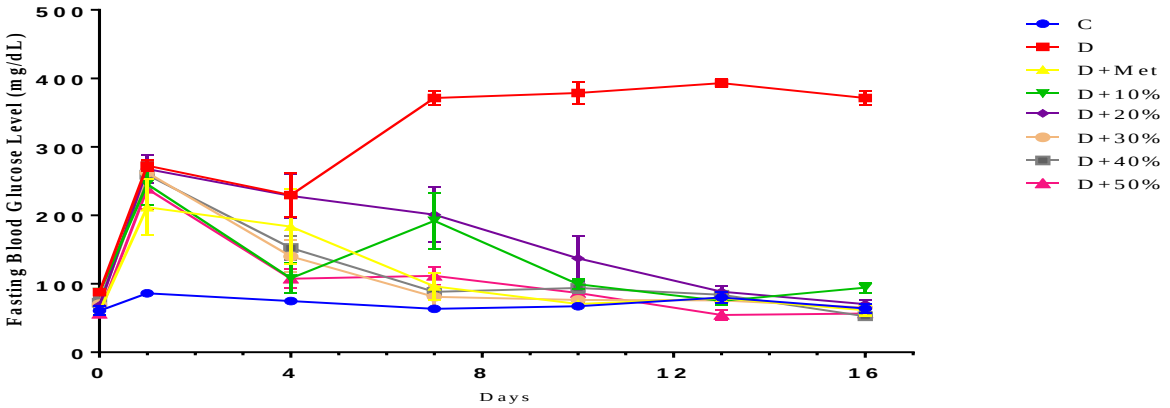

Figure 1: **Fasting Blood Glucose Concentration of Fructose and Streptozotocin-induced Diabetic Rats Treated with *F. exasperata* Leaf-based Diet**

*Values are means of 6 determinations ± S.E.M. *Day 0=FBG before diabetes induction; Day 1= FBG after diabetes induction*

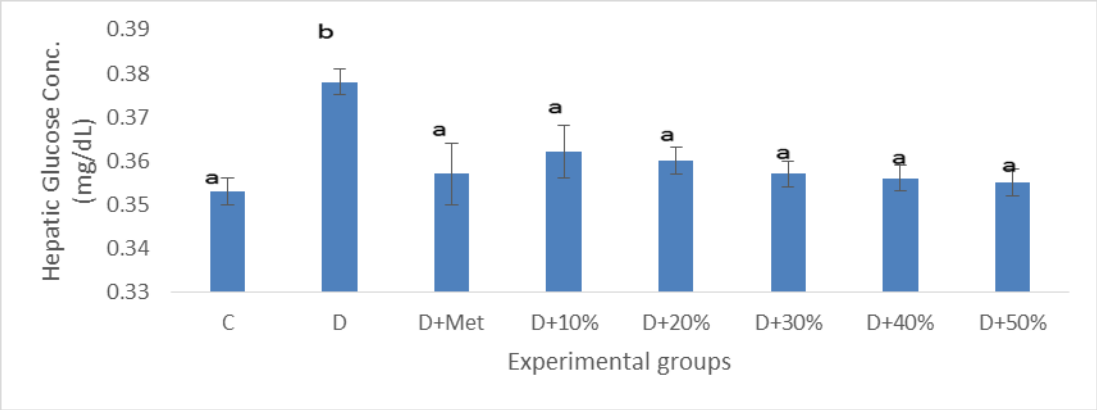

Figure 2: Hepatic Glucose Concentration of Fructose and Streptozotocin-induced Diabetic Rats Treated with *F. exasperata* Leaf-based Diet

Values are means of 6 determinations ± S.E.M. and bars with different superscripts are significantly different ($p < 0.05$)

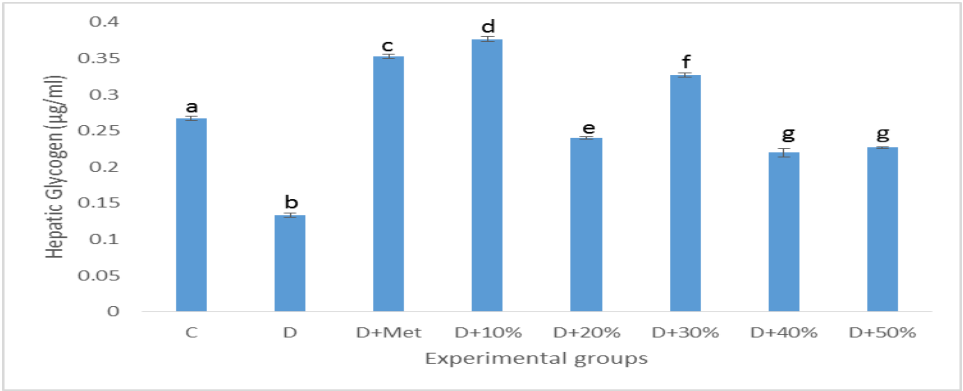

Figure 3: Hepatic Glycogen Level of Fructose and Streptozotocin-induced Diabetic Rats Treated with *F. exasperata* Leaf-based Diet

Values are means of 6 determinations ± S.E.M. and bars with different superscripts are significantly different ($p < 0.05$)

preparation such as fermentation, cooking, and malting increase the nutritive quality of plant foods through reducing certain antinutrients such as phytic acid, polyphenols, and oxalic acid (Hotz and Gibson, 2007).

Alteration in the metabolism of minerals had been reported in diabetes mellitus (Nielsen, 1990; Chausmer, 1998; Nerlich *et al*., 1998). Insulin action was reported to be potentiated by some trace elements like chromium, magnesium, vanadium, zinc, manganese, molybdenum and selenium (Candilish, 2000), which are present in the studied plant. The proposed mechanisms of enhancement of insulin action by trace elements include activation of insulin receptor sites (Vincent, 2000), serving as cofactors or components for enzyme systems which are involved in glucose metabolism (Waltr *et al*., 2003), increasing insulin sensitivity and acting as antioxidants for preventing tissue peroxidation (Kruse-Jarres and Rukguaer, 2000). The findings on the mineral composition in this study agree with those of Ajayi *et al*. (2012) and Bello *et al.* (2014). The nutrients composition of *F. exasperata* leaf suggests that it contained the macronutrients in significant amount as well as ash. Although, there was variations in the findings of this study in terms of concentrations with those of previous authors (Ajayi *et al*., 2012); Bello *et al*. (2014) ; Okenwa *et al.* (2015), the nutrients were within the range expected for dry leaf vegetable (Ajayi *et al*., 2012). Fibres present in FELD might be performing a function of slowing down the rate of glucose absorption into the blood, thereby reducing the risk of hyperglycemia (Bouttwell, 1998; Okeke and Adaku, 2009).

The significant increase in fasting blood glucose concentration of fructose and streptozotocin-induced diabetic rats as observed in this study confirmed induction of diabetes mellitus. However, upon feeding with FELD, the significant decrease in the concentration of FBG compared well with the negative control. By day 10 through day 16 of the experiment, a normoglycaemic effect was elicited by the diet containing 30%, 40% and 50% of *F. exasperata* leaf. The observed normoglycaemia was sustained for the group fed with 30% FELD throughout the 16 days of the experiment. The groups maintained on 40% and 50% FELD however became hypoglycaemic after day 10 of the experiment. This result suggests that 30%, 40% and 50% of FELD were efficacious in the treatment of hyperglycaemia in type 2 diabetes mellitus. This might be due to the presence of saponins, flavonoids, phenols, magnesium, zinc, chromium, fibre and ash (Waltr *et al*., 2003; Yu *et al*., 2003; Okeke and Adaku, 2009) in the formulated diet.

Glycosylated haemoglobin ($HbA_{1c)}$ of the diabetic animal was significantly higher compared to the normal control also confirmed diabetes induction. The non-significant difference of glycosylated haemoglobin of the treatment groups which compared well with the control at the end of the experiment suggests amelioration of hyperglycemia in the diabetic rats.

The non-significant difference observed in the serum insulin concentration for all diabetic animals when compared with the positive control further confirms induction of type 2 diabetes. Under normal physiological conditions, high blood glucose concentration promotes insulin release from the β-cells of the islets of Langerhans of the pancreas. Insulin stimulates the uptake of glucose by peripheral tissues especially skeletal muscle by up-regulating the expression of glucose transporter-4 (GLUT-4) and by stimulating the exocytosis of stored GLUT-4. The hormone also promotes the storage of glucose in the liver (as glycogen) through the stimulation of glycogen synthase activity (Zunino, 2009). Loss of responsiveness to insulin by insulin-responsive tissues results in the sustained elevation of blood glucose concentration (hyperglycemia), and ultimately to type 2 diabetes mellitus, a metabolic condition that affects the metabolism of carbohydrates, lipids and proteins (Pareek *et al.*, 2009).

In this study, the HOMA IR assessment after induction showed a significant increase in the diabeteic animals when compared with the control. At the end of the experiment, a significant reduction of HOMA IR in all the treatment groups compared with the negative control group was observed. This suggests improvement in insulin sensitivity by the peripheral tissues. Magnesium intake from foods has been previously reported to be protective against insulin resistance in non-diabetic

participants (Wang *et al*., 2013). Therefore, the magnesium content of FELD might have contributed to the observed effect. The HOMA β scores were reduced in the negative control group when compared with the control before the commencement of treatment. Homeostasic model assessment (HOMA IR) and HOMA-β scores are often used to validate insulin resistance and pancreatic β-cell function (Mattew *et al*., 1985; Song *et al*., 2007). This observation was however reversed at the end of the experiment.

In this study, there was a decrease in hexokinase activity of the diabetic untreated groups (i.e negative control) when compared with the control, suggesting a potential decrease in glycolysis in non insulin dependent diabetes mellitus (NIDDM). This was however ameliorated in the treatment groups where their hexokinase activity was not significantly different compared to the normal control.

The alteration that characterizes hepatic glucose and glycogen metabolism in untreated type 2 diabetic mellitus was reversed by FELD consumption in this study. The reduction in hepatic glucose concentration of the FELD treated groups may be due to inhibition of glucose 6 phosphatase activity and activation of pyruvate dehydrogenase activity in the liver. While the significant increase in the hepatic glycogen concentration of the FELD treated groups might be due to inhibition of hepatic glycogen phosphorylase thereby suppressing glycogeneolysis.

Conclusion

Thirty percent (30%) *Ficus exasperata* leaf-based diet consumed for 16 days elicited normoglycaemic effect in fructose and streptozotocin-induced diabetic rats. FELD could therefore be further explored in the development of food products for the management of hyperglycaemia in type 2 diabetes mellitus.

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