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Improvement of Oral Glucose Tolerance and Total Lipid Profile of Diabetic Rats Treated with *Ficus exasperata* Leaf-Based Diet

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***Abstract*:** The aim of this study was to investigate the effect of *Ficus exasperata* leaf-based diet (FELD) on oral glucose tolerance and total lipid profile of type 2 diabetic rats. Forty-eight wistar rats were randomly selected into 8 groups of 6 animals each. All experimental animals apart from the positive control group were administered with 10 % fructose solution ad libitum for 2 weeks, while those in the negative control group received distilled water. The remaining groups were diabetic rats treated with 10, 20, 30, 40 and 50 % FELD for 16 days. Oral glucose tolerance test (OGTT), cholesterol, triglycerides, high and low density lipoproteins (HDL and LDL) were assayed for. Result of OGTT before commencement of treatment showed significant increase ($p < 0.05$) in the blood glucose concentration after 30 mins of oral glucose load which was not restored back to the basal level after 2 hours. OGTT result by the 13th day of treatment showed significant decrease ($p < 0.05$) in glucose concentration of FELD-treated diabetic rats 2 hours after glucose load. There was a significant decrease ($p < 0.05$) in the concentration of cholesterol, triglycerides, LDL and a significant increase ($p < 0.05$) in HDL concentration of FELD-treated diabetic rats. In conclusion, incorporation of 30, 40 and 50 % *F. exasperata* leaf into diet and consumed for 16 days improved glucose tolerance and total lipid profile of diabetic rats.

***Keywords*:** Oral glucose tolerance, *Ficus exasperata* leaf-based diet, Total lipid profile, Diabetes mellitus, streptozotocin

Introduction

Diabetes mellitus is a metabolic disorder characterized by persistent elevated blood glucose level and alteration in the metabolism of carbohydrates, proteins and Lipids in the body. Diabetes mellitus is a major public health problem [1, 2]. It remained one of the leading cause of death

worldwide, resulting in 1.5 million deaths (43 %) of people aged under 70 years in year 2012 [2]. In 2014, 422 million people in the world had diabetes, a prevalence of 8.5% among the adult population [2]. Complications arising from untreated diabetes mellitus include retinopathy, nephropathy, neuropathy and atherosclerosis [2].

In Africa, about 25 million people aged 18 and above had diabetes in 2014 with a prevalence of 7.1 % [2]. According to report of International diabetes Federation, there were more than 1.56 million cases of diabetes in Nigeria in 2015.The total adult population (1000s) aged 20-79 yrs were 82,868 and number of death in adult due to diabetes was 40,815. Prevalence of diabetes in adult (20 – 79 yrs.) was 1.9 % and cost per person with diabetes (USD) was 212.3. Total cases of adults (20-79 years) with diabetes (1000s) was 1,564.7. Number of cases of diabetes in adults that are undiagnosed (1000s) was 949.9 [3].

Over the years, the use of herbal plants to treat different diseases have also been adopted across the continents. Plants have been recognized as potential sources of therapeutic agents against various diseases due to their biodiversity and presence of a wide array of bioactive phytochemicals and secondary metabolites [4]. Several investigations into the chemical and biological activities of plants have yielded compounds with properties useful for the development of modern synthetic drugs for management of several diseases including diabetes [5, 6, 7, and 8]. One of such plant that has been variously studied for its usefulness in managing a wide array of medical conditions is *Ficus exasperata.*

F.exasperata 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* leaves traditionally for the management, control and/or treatment of an array of human diseases, including diabetes mellitus and hypertension. Various pharmacological actions such as anti-diabetic, lipid lowering and antifungal activities have been reported for *F.exasperata* [9, 10].

The use of medication is vital in the management of T2DM. However, the effectiveness of the treatment is largely dependent on the level of adherence toward prescribed medication [11, 12, 13]. Hence, the use of a diet based therapy is expected to improve compliance and adherence to drug use by patients by providing a psychological feeling in the users that they are feeding on food rather than drug [9, 14, 15, 16].

The aim of this study was to investigate the effect of *Ficus exasperata* leaf-based diet on oral glucose tolerance and total lipid profile of type 2 diabetic rats.

Material and Methods

Materials

Plants collection and Authentication

Ficus exasperata leaf was collected within Ilorin community, Nigeria. It was identified and authenticated at the herbarium unit of the department of Plant Biology, University of Ilorin, Ilorin, Nigeria and a voucher number, UILH/001/883 was issued.

Experimental animals

Wistar o rats of Norvegicus strain weighing approximately 150 g was

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 for 7 days. They were fed with normal rat pellet and tap water during this period.

Ethical Clearance

Ethical clearance for this study and on animal handling was obtained from the University of Ilorin ethical review committee (UERC). Protocol identification code UERC/LSC 067 and UERC approval number UERC/ASN/2016/243 was obtained.

Drugs and Chemicals

Streptozotocin was a product of Sigma-Aldrich, St. Louis, MO, USA and metformin was a product of Austell Laboratories Pvt. Ltd., Johannesburg, South Africa. Fructose was a product of Nature's Choice™ Wholefood specialists, Meyerton, South Africa.

Cholesterol, triglycerides and HDL kits were product of Fortress Diagnostics Ltd., Antrim Technology Park, Antrim BT41 1 QS, United Kingdom. All other chemicals and reagents were of analytical grades and prepared in all-glass apparatus using distilled water.

Feed material

Yellow corn, Maize husk, Soy bean oil, Soy bean grain and sucrose (sugar) were purchased from Oja tuntun and Olufadi market in Ilorin, Kwara State, Nigeria. Vitamin and mineral mix, DL-Methionine, Fructose and L- Lysine were purchased from Aromokeye store, Ilorin, Kwara State, Nigeria.

Methods

Plant Processing

Fresh leaves of *F. exasperata* were collected and air dried to a constant weight and pulverized into fine powder using an electronic blender, stored in air tight container and kept in a refrigerator prior to analysis.

Induction of Type 2 Diabetes Mellitus

All experimental animals apart from animals in the positive control group were administered with 10% fructose solution ad libitum for 2 weeks, while those in the positive control group were given distilled water. Streptozotocin (STZ) was then dissolved in 0.1 M citrate buffer (pH 4.5). At the end of the 2 weeks administration of fructose solution, animals were fasted overnight and each of the fructose-fed animals were injected (ip) with a low dose STZ (40 mg/kg b.w.) [17].

Confirmation of Type 2 diabetes

One week after the STZ injection, fasting blood glucose level of the animals were checked using AccuChek active glucometer and compatible strips by withdrawing blood from the caudal vein of the rats' tail. Rats showing glucose concentration above 125 mg/dL were considered diabetic.

Feed Preparation and Administration to Experimental Rats

Corn starch was prepared by rinsing and soaking 10 kg of yellow corn in 20 litres of distilled water for 72 hours, followed by grinding and sieving. The filtrate was drained for 6 hours and oven-dried at 40 ^{o}C to constant weight. Ten kilograms of maize husk was sun-dried for 3 days and pulverized using commercial grinder. Soy bean grain (7 kg) was soaked in 15 litres of distilled water for 6 hours and the seed coats removed. Thereafter, it was sun-dried for 3 days and ground to smooth texture.

Corn starch, maize husk (cellulose source), sucrose, ground soybean,

vitamin/mineral mix, D- methionine and L-lysine were thoroughly mixed together in the various proportion indicated in Table 1. Soybean oil (40 ml) and distilled water (1000 ml) were added slowly to the mixed ingredients until the mixture became a paste. The paste was then grated on a wire mesh to form pellets which were oven-dried at 40 ^{o}C to a constant weight. Formulated feed was administered to experimental animals ad libitum for a period of 16 days.

Table 1: Feed Formulation Using *F. exasperata* Pulverized Leaf

Ingredients g/kg	+ve Control	-ve Control	Reference drug	10%	20%	30%	40%	50%
Corn starch	512	512	512	412	312	212	112	12
Cellulose	40	40	40	40	40	40	40	40
Sucrose	100	100	100	100	100	100	100	100
Soybean	250	250	250	250	250	250	250	250
Soybean Oil	40	40	40	40	40	40	40	40
Vitamin/mineral mix	50	50	50	50	50	50	50	50
D- Methionine	4	4	4	4	4	4	4	4
L-lysine	4	4	4	4	4	4	4	4
F. exasperata leaf	-	-	-	100	200	300	400	500

Percentage Nutrient composition: Carbohydrate - 65.2%; Protein - 25.8%; Fats - 4%; Vitamin & minerals - 5 %

Experimental Design

Forty-eight (48) wistar rats were randomly selected into 8 groups of 6 animals each namely; Control (C), these were non diabetic rats fed with feed without *F. exasperata* leaves. Diabetic untreated (D), these were diabetic rats

also fed with formulated feed without *F. exasperata* leaves. D + Met, diabetic rats treated with 12.14 mg/kg b.wt. standard drug, metformin. D + 10 %, diabetic rats fed with feed containing 100 g/kg of *F. exasperata* leaves. D + 20 %, diabetic rats fed with feed containing 200 g/kg of *F. exasperata* leaves. D + 30 %, diabetic rats fed with feed containing 300 g/kg of *F. exasperata* leaves. D + 40 %, diabetic rats fed with feed containing 400 g/kg of *F. exasperata* leaves. D + 50 %, diabetic rats fed with feed containing 500 g/kg of *F. exasperata* leaves.

Experimental rats were then sacrificed 24 hours after the last day of treatment. They were anaesthetized with diethyl ether and sacrificed by incising the jugular vein using a scalpel. Blood samples were collected into plain sample bottles for serum collection. Serum was collected by allowing blood sample to stand at room temperature for 30 minutes to form clot. The supernatant which is the serum was collected using a Pasteur pipette.

Biochemical Analysis

Oral Glucose Tolerance Test

Oral glucose tolerance test was carried out using the method described by [18]. Rats were fasted overnight and given an oral glucose load of 2 g/kg body weight. Following the oral glucose load, blood was obtained at 0, 30, 60, 90 and 120 minutes from the tail vein of the rat and analysed for glucose concentration using a glucometer (Accu-check Advantage, Roche Diagnostics, Mannheim) as described for FBG quantification. The procedure was carried out on day 1, 3 and 13.

Serum Lipid Profile determination

The method described by [19] was employed in the determination of cholesterol concentration. HDL concentration was determined using the method described by [20]. Triglycerides concentration was determined using the method described by [21]. The method described by [22] was employed for the determination of LDL concentration.

Statistical Analysis

All data were expressed as mean of six replicates ± standard error of mean (S.E.M). Statistical evaluation of data was performed by SPSS version 16.0 using one way analysis of variance (ANOVA), followed by Duncan's multiple range test for multiple comparison. Values were considered statistically significant at $P < 0.05$ (confidence level = 95%).

Results

Oral Glucose Tolerance in Diabetic Rats Fed with *Ficus exasperata* Leaf-based Diet

The results of oral glucose tolerance test are presented in Figures 1, 2 and 3. Figure 1 showed the result of the oral glucose tolerance after confirmation of the diabetes induction, before commencing on treatment. Result showed there was a significant increase ($p < 0.05$) in the blood glucose concentration and the increase did not return to basal level after 2 hours of the glucose load. Figures 2 and 3 showed the results of the oral glucose tolerance test done on day 3 and 13 of the experiment after commencement of treatment. The results showed significant reduction ($p < 0.05$) in the blood glucose concentration of the rats which returned to basal level by day 13 of the experiment (Figure 3).

Serum Total Lipid Profile in Diabetic Rats Fed with *Ficus exasperata* Leaf-based Diet

Figure 4 the serum total lipid profile in diabetic rats fed with FELD. Result shows a significant decrease ($p < 0.05$) in the concentration of cholesterol, triglycerides and low density lipoprotein cholesterol (LDL) in the FELD treated groups compared to the negative control and no significant difference ($p < 0.05$) when compared to the positive control. However, HDL concentration was significantly increased in the treatment groups compared to the negative control (D) but not as high as the positive control (C).

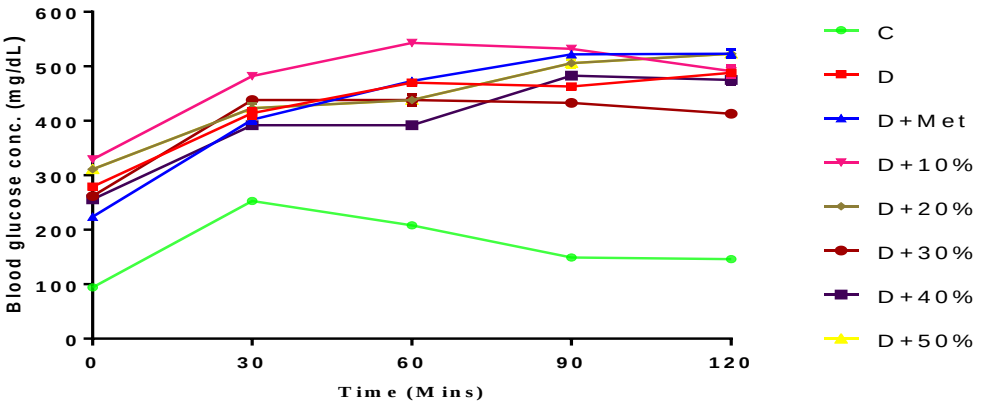

Figure 1: Oral Glucose Tolerance Test of Fructose and Streptozotocin-induced Diabetic Rats Fed with *F. exasperata* Leaf-based Diet on Day 1 Pre-treatment
Values are expressed as mean of 6 replicates ± S.E.M.

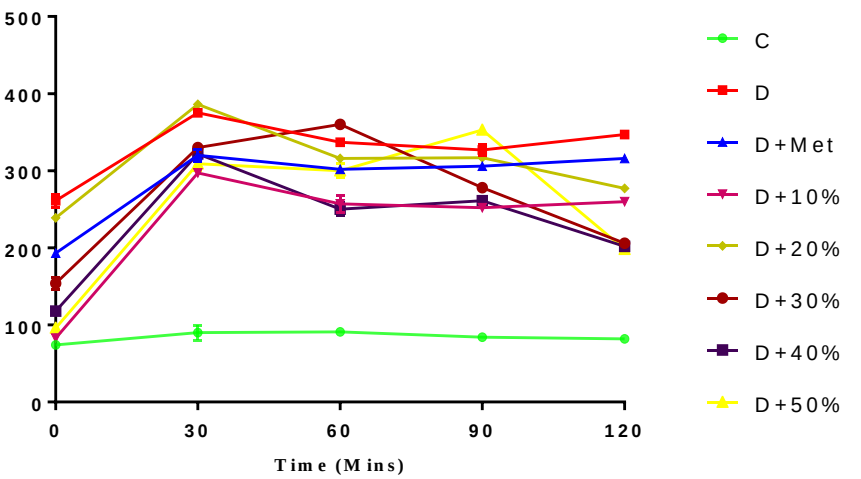

Figure 2: Oral Glucose Tolerance Test of Fructose and Streptozotocin-induced Diabetic Rats Fed with *F.exasperata* Leaf-based Diet on Day 3 of Treatment
Values are expressed as mean of 6 replicates ± S.E.M.

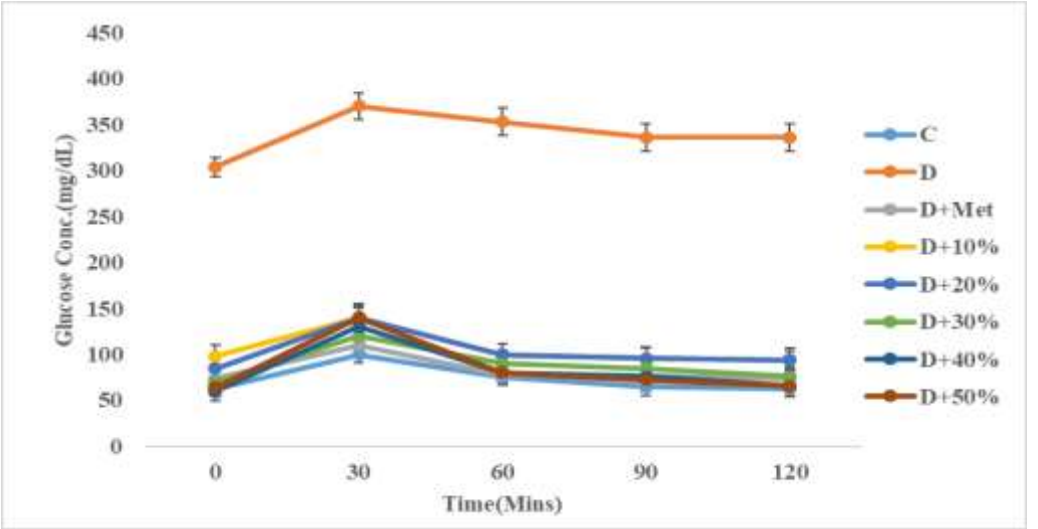

Figure 3: Oral Glucose Tolerance Test of Fructose and Streptozotocin-induced Diabetic Rats Fed with *F.exasperata* Leaf-based Diet on Day 13 of Treatment

Values are expressed as mean of 6 replicates ± S.E.M.

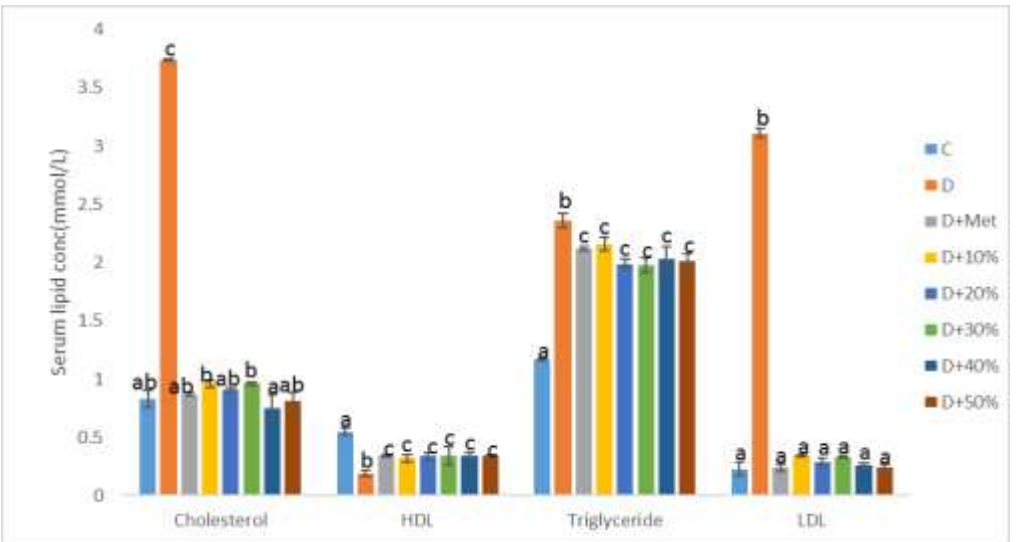

Figure 4: Total Lipid Profile of Fructose and Streptozotocin-induced Diabetic Rats Fed with *F. exasperata* Leaf-based Diet

Values are expressed as mean of 6 replicates ± S.E.M. and those with different alphabets are statistically different (P < 0.05)

Discussion

Figure 1 shows the result of the oral glucose tolerance test on the diabetic animals before the commencement of treatment. The blood glucose concentration of all the experimental groups increased significantly ($P < 0.05$) after 30 mins of oral glucose load. Throughout the two hours of the test, glucose concentration for all the diabetic animals was not restored back to the basal glucose concentration. This suggests possible impairment in the utilization of glucose by the animals. Typically, type 2 diabetes is characterized by insulin resistance and hyperglycemia. After glucose absorption, the peripheral tissues could not fully utilize glucose due to loss of sensitivity to the action of insulin (glucose utilization promoter), hence, resulting in the sustained steady rise in the concentration of blood glucose during the 2 hour period after glucose load in the animals. The oral glucose tolerance test (OGTT) measures an individual organism's ability to utilize ingested glucose, the body's main source of energy, over a given period of time [23, 24, 25].

Figure 2 and 3 shows the result of oral glucose tolerance test of the experimental animals by the 3rd and 13th day of treatment. The blood glucose concentration of all the diabetic animals treated returned back to the basal glucose concentration apart from diabetic untreated groups that remained steadily high. This result therefore, shows that F. exasperata leaf-based diet improved oral glucose utilization using oral glucose tolerance test. Literature had shown that F. exasperata leaf contain certain phytochemical such as saponins, flavonoids, tannins, terpenoids and phenolics [26, 27, 28]. Some of these constituents have been reported to possess antihyperglycemic and antihyperlipidemic effects.

Although literature has shown that F. exasperata leaf extract possesses antihyperglycemic effects [10], the results of this study has also shown that treating diabetic animals with F. exasperata leaf through diet also improved glucose tolerance for diabetic animals fed with 30, 40 and 50 % Ficus exasperata leaf-based diet. Diet based therapy is expected to improve compliance and adherence to drug use by patients by providing a psychological feeling in the users that they are feeding on food rather than drug [9, 14, 15, 16].

Hypertriglyceridemia is one of the major abnormalities found in diabetes with insulin deficiency [29]. Insulin inhibits the activity of hormone-sensitive lipase in the adipose tissue, thus reducing the release of free fatty acid and glycerol [30]. The deficiency of insulin in diabetes mellitus therefore causes excessive mobilization of chylomicrons and VLDL leading to hypertriglyceridemia [30]. Result of the total lipid profile (Figure 4) showed a significantly high ($P < 0.05$) level of cholesterol, triglycerides and low density lipoprotein cholesterol (LDL) of animals in the negative control group (D) compared to positive control (Figure 4). Upon treatment with F. exasperata leaf-based diet, there was a significant decrease ($P < 0.05$) in the concentration of these parameters for all the treatment groups compared to the negative control and no significant difference when compared to the positive control. This finding is in

agreement with reports of previous researchers suggesting that aqueous leaf extract of Ficus exasperata could ameliorate diabetic dyslipidemia [10, 31].

In conclusion, incorporating 30, 40 and 50 % F. exasperata leaf into diet and consumed for 16 days improved glucose tolerance favorably compared to the healthy animals (positive control) and total lipid profile of diabetic rats. The improvement in the lipid profile was not as low as the positive control.

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