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The antidiabetic effect of the lime juice (citrus aurantifolia) extract of Ficus exasperata in streptozotocin-induced rats

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ABSTRACT

Background: Diabetes is a chronic incapacitating illness that imposes a huge burden on global healthcare. The leaves of *Ficus exasperata* soaked in lime juice are adopted in folkloric unconventional medicine to treat diabetes. This study was aimed at determining the protective effect of lime juice extract of *Ficus exasperata* (LFE) in streptozotocin (STZ)-induced diabetes mellitus in rats.

Methods: LFE (50, 250, or 500mg/kg) or vehicle was administered before the oral glucose tolerance test (OGTT), insulin tolerance test (ITT), or after establishing diabetes mellitus in the STZ model, in rats. One-hour post-treatment on days 7 and 14, fasting blood glucose and weight were recorded, which was followed by assessment of biochemical, antioxidant and histological parameters.

Results: LFE produced dose-dependent and significant control of glycemic index with peak effect at 500mg/kg in OGTT and ITT when compared with vehicle treated control. In addition, LFE also reversed STZ-induced hyperglycemia and oxidative stress in selected organs when compared with vehicle-treated control group. Similarly, STZ-induced hepatic damage, anemia, and immunosuppression were also reversed by LFE administration.

Conclusion: Findings from this study showed the beneficial effect of lime juice extract of *Ficus exasperata* in the management of diabetic mellitus through increase in antioxidant defense signaling

1. Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by chronic hyperglycemia and hyperlipidemia, resulting from defects in insulin secretion, insulin action, or both, thus causing a concomitant increase in both fasting and postprandial blood glucose levels[1]. This disease imposes a huge economic burden on the global healthcare system due to its debilitating and long-term complications thus often termed the 'silent epidemic'. The condition has shown a tremendous increase in prevalence with a burden measured through direct and indirect medical costs incurred on the affected individual. This is further exacerbated by the rising poverty levels with over 83 million Nigerians reported living below the poverty line (less than $1 per day) as reported by the Nigerian National Bureau of Statistics in 2020[2].

A meta-analysis by Uloko *et al*[3] reported that about six

million Nigerians are living with DM. The prevalence of undetected diabetes approximated about two-thirds of cases in Nigeria, a scenario likened to the thin "edge of a wedge" as this value keeps escalating to a conundrum for resource- limited nations[4]. This development observed more in developing countries of the Sub-Saharan Africa (SSA) region has not only resulted in the hindrance of a good prognosis of diabetes viz a viz complications and deaths but has also put a remarkable strain on the frail health system in this sub-region. The total health expenditure on diabetes more than tripled which spanned the period of 10 years (2003 -2013)[5]. In 2019, there were an estimated 463 million DM cases globally, and this value is expected to accelerate to 578 million by 2030[6]. The current global focus to repress premature mortality from Non-Communicable Diseases by 33 %, as a part of the 2030 Agenda for Sustainable development to achieve universal health coverage and provide access to affordable medicine has been set[7].

It is noteworthy that since time immemorial, plants have been used for the management of diabetes mellitus all over the world, especially in Asia and Africa[8]. In folklore medicine, various parts of *Ficus exasperata* Vahl. (*Moraceae*) are used in the treatment of various ailments including hypertension, ulcer, and diabetes mellitus[9,10].

The maceration of *F. exasperata* leaves in lime juice is used in Traditional African Medicine for the treatment of diabetic mellitus. Although, different fractions of the leaves have shown the potential anti-diabetic property[11]. Several studies have shown a reduction in the postprandial blood glucose rise in the aqueous extract but not the lime juice extract. This present study sought to determine the beneficial effect of the lime juice extract of *Ficus exasperata* in streptozotocin-induced diabetic mellitus in rats.

2. Materials and Methods

2.1 Collection of plant materials and extraction

The fresh leaves of the plant *Ficus exasperata* were collected from Akure Forest Reserve, Ondo State, Nigeria (Latitude of 7° 17′ 39″ N and Longitude of 5° 2′ 3″ E). It was identified and authenticated by a botanist and was given an herbarium voucher specimen number -LUH 7499 for reference purposes. Air-dried pulverized leaves (1.5 kg) of *ficus exasperata* were soaked in 4.2 L of lime juice (c*itrus aurantifolia*) for 72 h with intermittent shaking at 4°C. After 72 h of soaking, it was decanted and filtered, with the filtrate freeze-dried to give a golden brown solid preserved at 4°C. A freshly reconstituted extract in distilled water was made before each administration to experimental rats.

2.2.2 Laboratory Animal

Healthy mice (20-25 g) and rats (170-200 g) of both genders were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos and kept under standard environmental conditions (12 hours light and 12 hours dark cycle), the animals were nurtured with free access to standard rodent feed and water. They were fasted overnight but had access to water, prior to the commencement of the experiments. The study was approved by the College of Medicine, the University of Lagos, Health Research Ethics Committee (CM/HREC/10/17/450) which is in agreement with the United States National Institutes of Health guidelines for the care and use of laboratory rats for biomedical research[12].

2.2.3 Acute toxicity study

Acute oral toxicity studies of the LFE were conducted using the limit dose procedure of OECD test guidelines on acute oral toxicity. Briefly, the extract up to 2000 or 5000 mg/kg, respectively, were administered intraperitoneally and orally. The behavioral toxic effect (such as hyperactivity, hyperventilation, diarrhea, urination, and calmness) was recorded for the first 3 h and the number of deaths for the first 24 h followed by 14 days.

2.2.4 Phytochemical analysis of LFE

The qualitative phytochemical analysis of LFE was analyzed using the earlier reported protocol of Sofidiya *et al*[13].

2.2.5 LFE on oral glucose tolerance test (OGTT) in normal rats

Thirty albino rats fasted overnight were randomly allotted into six groups (n=5) and treated orally as follows; Group I - vehicle 10ml/kg, normal control; Group II- Vehicle 10ml/kg, Group III –V: LFE (50, 250 or 500mg/kg, respectively), Group VI- LFE (500mg/kg). One-hour post-treatment, 3g/kg, p.o. of glucose D was administered to rats in groups II-V. Time course change in blood glucose level was determined at baseline, 0, 15, 30, 60, 90, and 120 min.

2.2.6 LFE on insulin tolerance test

Thirty albino rats fasted overnight were randomly divided into six groups (n=5) and treated orally as follows; Group I -

vehicle 10ml/kg, normal control; Group II- Vehicle 10ml/kg, Group III –V: LFE (50, 250 or 500mg/kg, respectively), Group VI- LFE (500mg/kg). Fifteen minutes post-treatment, insulin (0.75U, i.e.) was administered to rats in group II-V. Determination of blood glucose levels was done through the tail vein at 15, 30, 60, 90, and 120 min.

2.2.7 Streptozotocin -induced type 2 diabetes mellitus in rats

Experimental rats were assigned into vehicle-control group (10 ml/kg, n=8) or LFE (500mg/kg, non-diabetic rats), diabetes mellitus group (DM group, n=8), and LFE treatment groups (DM + LFE 50, 250 or 500mg/kg, respectively, n=8 per group) or metformin (500mg/kg). Streptozotocin (STZ) was dissolved in citrate buffer (0.1 M, pH 4.5) and administered intraperitoneally to establish diabetes. To confirm experimental diabetes, blood was taken from caudal vein to test glucose level and rats with the glucose level of ≥ 16.7 mmol/l were deemed as a suitable diabetic model and chosen for further experiment. Clinical manifestations of DM in rats were observed and recorded every day. In the DM model, rats were intragastrically fed with LFE 50, 250 or 500mg/kg/day, or metformin (500mg/kg) for 2 weeks. The control groups were treated with vehicle or LFE (500mg/kg/day) for 2 weeks. One-hour post-treatment on day 14, rats were anaesthetized, blood samples collected and vital organs (liver, kidney, and pancreas) were harvested for biochemical studies.

2.2.8 Biochemical assay

The measurement of lipid peroxidation was determined by measurement of malondialdehyde (MDA) using a protocol by Buege and Aust[14], redox potential of reduced glutathione (GSH) was ascertained spectrophotometrically using a method by Ellman[15] while the catalase (CAT) activity was demonstrated colourimetrically according to the method of Aksenes and Njaa[16]. Finally, the superoxide dismutase (SOD) activity was established as described by Sun and Zigma[17].

2.2.9 Antioxidant activity

In vitro antioxidant activity was carried out on the lime juice extract of *ficus exasperata* by testing the reducing power antioxidant activity, nitric oxide scavenging activity, and DPPH scavenging activity. The IC_{50} was also determined.

2.2.10 Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). For comparison of the mean values among the multiple groups, data were analyzed using one or two-way ANOVA followed by Tukey's *post hoc* test multiple comparison tests using Graph-Pad Prism 6 (Graph-Pad Software Inc., California, United States of America). Statistical significance was considered at $p < 0.05$.

3.0 Results

3.1 Qualitative phytochemical analysis

The preliminary phytochemical analysis revealed the presence of alkaloids, steroids, saponins, flavonoids, tannins, reducing sugar, and terpenoids.

3.2 Acute toxic effect of LFE

The LFE up to a dose of 5000 mg/kg, is non-toxic, as no mortality was recorded. The extract did not produce any toxicity behavior up to 14 days of observation.

3.3 LFE prevented oral glucose load-induced hyperglycemia

OGTT is the gold standard for the diagnosis of diabetes, in this study, oral glucose load of 3g/kg significantly increased venous plasma glucose as shown in table 1. However, the pretreatment of rats with LFE (50, 250 or 500 mg/kg) produced time course and dose dependent decrease in plasma glucose level.

Table 1: Effect of lime juice extract of *Ficus exasperata* on oral glucose tolerance test in rats

	Time in minutes					
Group	0	15	30	60	90	120
Normal	65.80 ± 4.10	62.00 ± 2.12	60.40 ± 5.11	79.20 ± 10.35	58.60 ± 2.50	58.40 ± 3.30
Glucose	67.60 ± 6.21	100.80 ± 7.42	101.40 ± 6.10	97.80 ± 6.43	81.60 ± 7.99	76.80 ± 3.93
LFE 50mg/kg	73.60 ± 5.53	114.20 ± 8.48	103.40 ± 6.34	91.40 ± 8.98	73.20 ± 6.38	76.60 ± 8.86
LFE250mg/kg	79.60 ± 6.75	96.20 ± 10.17	105.40 ± 6.11	89.60 ± 5.43	63.40 ± 3.61	65.20 ± 1.80
LFE500mg/kg	63.80 ± 3.76	112.20 ± 5.34	81.60±5.27 ****	96.00±3.86 ***	62.60 ± 9.14*	58.20 ± 1.71
LFE 500m/kg Only	56.80 ± 3.79	59.60 ± 2.60	59.20 ± 5.76	58.20 ± 5.71	54.80 ± 5.95	52.60 ± 4.11

Values are mean ± SEM (n=5) *p<0.05, ***p<0.01, ****p<0.001 versus control. Statistical level of significance by two-way ANOVA followed by Bonferonni post hoc multiple comparison test.

3.4 LFE ameliorates STZ-induced hyperglycemia in rats

Seventy-two hours after STZ administration, fasting blood glucose levels in all DM groups were significantly higher compared to the control group (P < 0.001) (Fig. 1A). Two weeks after administration of LFE or metformin, the blood glucose levels significantly reduced compared to those in vehicle control diabetic group.

3.5 LFE sensitivity to insulin challenge

ITT is a simple and reliable assay of assessing insulin sensitivity. Intraperitoneal injection of insulin produced time course and significant decrease in mean fasting blood glucose level when compared with vehicle treated control. However, LFE 500mg/kg significantly reversed the effect of insulin (Fig. IB).

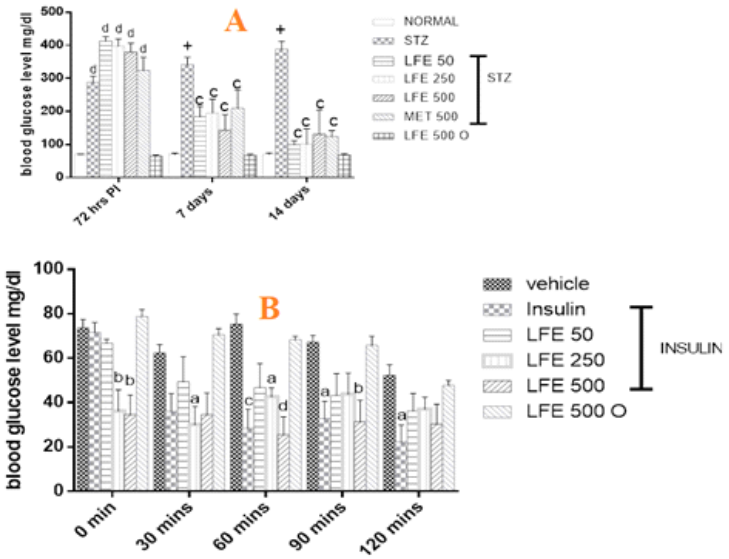

Figure 1 A-B: Effect of LFE on blood glucose levels in (A) insulin tolerance test in rats and (B) oral glucose tolerance test in rats. Values are mean ± SEM (n=5) ap<0.05, bp<0.01, cp<0.001 versus control.

3.6 Effect of LFE and STZ on liver, kidney and pancreas weights

STZ significantly reduced body weight 7 days post exposure in comparison to normal control. However, STZ-induced weight loss was reversed dose dependently and significantly by LFE administration (Table 2). Conversely, we do not observe any significant change in organ weights (liver, kidney and pancreas when compared with normal control (data not shown).

Table 2: Effect of lime juice extract of *Ficus exasperata* on body weight

Group	72 hrs. post-induction	7 days post-treatment	14 days post-treatment
Normal	102.60 ± 1.17	110.80 ± 0.97	113.80 ±1.11
STZ 60mg/kg	87.00 ± 5.59	91.00 ± 5.30 ^{d}	97.00 ± 2.00 ^{d}
LFE 50mg/kg	89.40 ± 4.93	102.40 ± 4.23	107.80 ± 3.18
LFE250mg/kg	97.20 ± 1.98	107.25 ± 5.02 **	110.67 ± 5.21 **
LFE500mg/kg	89.00 ± 1.22	120.00 ± 8.51 ****	120.67 ± 15.51 ****
MET 500mg/kg	83.60 ± 5.91	101.00 ± 4.85	105.00 ± 5.00
LFE 500m/kg	105.60 ± 1.03	107.80 ± 2.96	106.80 ± 1.39

Values are mean ± SEM. (n = 5) ^{d}p <0.0001 versus normal control, **p<0.01, ****p<0.0001 versus STZ control. The results were analyzed by two-way ANOVA followed by Tukey's post hoc test multiple comparison tests.

3.7 *F. exasperata* ameliorates STZ-induced oxidative stress in the pancreas

Post hoc test revealed the significant effect of STZ and LFE treatments on antioxidant enzyme activities. Administration of LFE reversed STZ-induced GSH deficit in the pancreas (Fig.2A), but not on SOD activity in the pancreas when compared with STZ-vehicle treated group (Fig. 2B). In contrast, STZ significantly reduced catalase activity in the pancreas in comparison to control. STZ-induced catalase deficit was significantly reversed by LFE or metformin treatment (Fig.2C). Conversely, STZ caused no significant change in MDA level (marker for lipid peroxidation). It remains unchanged across all treatment groups (Fig. 2D).

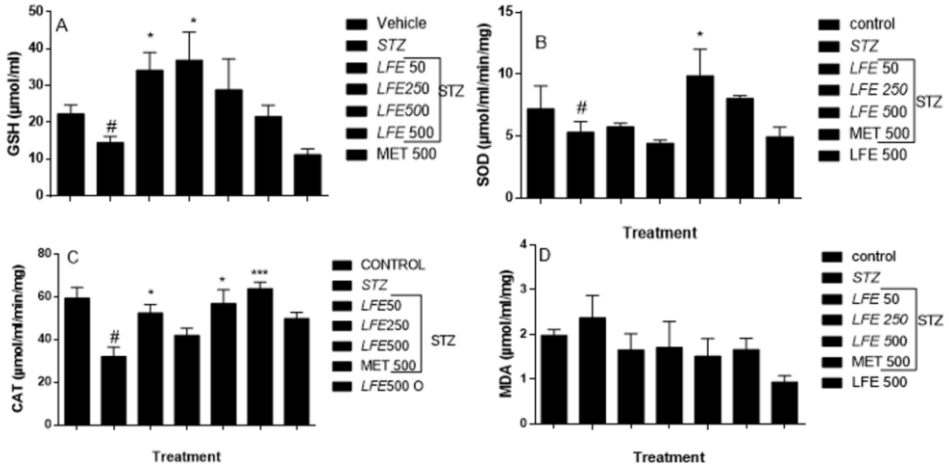

Figure 2A-D: Effect of LFE on STZ-induced oxidative stress (A) GSH level, (B) SOD activity, (C) catalase activity (CAT), (D) MDA level in the liver. Values are expressed as mean ± SEM. (n = 5) #p<0.05, versus control-treated group; *p<0.01, ***p<0.001 versus STZ-control treated . The results were analyzed by one -way ANOVA followed by Tukey's post hoc test multiple comparison

3.8 LFE attenuates STZ-induced oxidative stress in the liver

Post hoc test revealed the significant effect of STZ and LFE treatments on antioxidant enzymes. Administration of LFE GSH level in the liver (Fig.3A), in contrast, STZ-induced significant decrease in SOD activity in the liver when compared with normal control was reversed by oral administration of LFE 500mg/kg (Fig. 3B). Similarly, STZ significantly reduced catalase activity in the liver in comparison to normal control. STZ-induced catalase deficit was significantly reversed by LFE 500mg/kg administration (Fig. 3C). Moreso, STZ -induced increase in lipid peroxidation (significant increase in MDA level) was attenuated by metformin treatment (Fig. 3D).

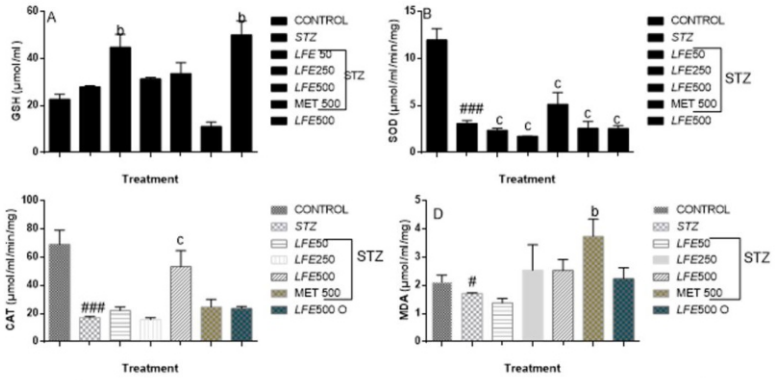

Figure 3A-D: Effect of LFE on STZ-induced oxidative stress (A) GSH level, (B) SOD activity, (C) catalase activity (CAT), (D) MDA level in the liver. Values are expressed as mean ± SEM. (n = 5) #p<0.05, ###p<0.001 versus control-treated group; bp<0.01, cp<0.001 versus STZ-control treated . The results were analyzed by one-way ANOVA followed by Tukey's post hoc test multiple comparison tests.

3.9 LFE reversed STZ-induced liver and kidney injury

The exposure of rats to STZ caused significant increase in markers of liver injury (ALT and ALP), creatinine clearance (34.94±1.07 to 49.38±1.37 in control) and blood cholesterol level, triglyceride when compared with vehicle control. However, post STZ exposure of rats to LFE significantly reversed STZ-induced liver and kidney injury as well as increase in cholesterol catabolism (Table 3).

Table 3: Effect of lime juice extract of *Ficus exasperata* on biochemical parameters

Parameters	Control (10 ml/kg)	STZ (60 mg/kg)	LFE (50 mg/kg)	LFE (250 mg/kg)	LFE (500 mg/kg) + STZ (60 mg/kg)	Metformin (500 mg/kg)	LFE (500 mg/kg)
ALT	33.77±6.13	93.90±1.00****	70.27±2.56[a]	100.2±0.88	42.93±1.66[d]	60.00±11.30[b]	46.23±4.32[d]
UREA	6.13±0.24	6.85±0.85	6.24±0.91	6.10±0.10	5.73±0.58	8.13±0.74	4.70±0.30
TP	75.38±2.75	70.50±4.60	69.56±2.72	74.00±2.00	64.98±2.35	57.77±4.15	73.98±2.77
HDL	0.11±0.02	0.08±0.02	0.08±0.01	0.54±0.46	0.08±0.01	0.08±0.01	0.13±0.03
LDL	0.27±0.06	0.13±0.05	0.14±0.02	0.20±0.01	0.09±0.02	4.77±4.61	0.27±0.06
CREAT	19.38±1.37	34.94±1.07***	40.23±1.10	40.21±1.00	29.38±1.29	38.71±2.13	43.31±1.78[a]
ALP	183.80±3.28	538.30±25.75****	461.80±30.25	468.20±2.00	160.20±10.29[d]	333.70±19.23[d]	119.60±6.91[d]
ALB	35.70±1.95	24.15±0.75	31.38±2.46	34.10±1.00	30.10±1.84	18.30±4.70	34.75±2.20
CHOL	1.79±0.34	1.43±0.16	1.17±0.10	1.97±0.01	1.05±0.10	1.23±0.06	1.87±0.26
TRIG	0.60±0.08	1.46±0.05***	0.79±0.06[b]	2.56±0.01[c]	0.71±0.06[b]	0.81±0.14[b]	1.06±0.16
VLDL	0.36±0.10	0.66±0.02	0.35±0.03	1.16±0.02[b]	0.32±0.03[a]	0.36±0.06	0.42±0.08

Data represented as mean ± S.E.M (n=5). ***p<0.001, ****p<0.0001 compared to control; [a]p<0.05, [b]p<0.01, [c]p<0.001, [d]p<0.0001 STZ-control treated. One-Way ANOVA followed by Tukey's multiple comparison test.

4.0 Discussion

Findings from this study showed the beneficial effect of F. exasperata on glucose and STZ-induced hyperglycemia as well as the ability to reverse insulin-induced hypoglycemia suggestive of insulin sensitivity. Moreso, the extract reversed the liver and kidney injury induced by STZ possibly through enhancement of antioxidant defense mechanisms owing to its richness in polyphenolic compounds.

Diabetes is a life- limiting deleterious condition with long-term microvascular complications ranging from diabetic neuropathy, with an increased risk of foot ulcers that can warrant the need for a foot amputation, diabetic nephropathy, retinopathy, and vasculopathy depicting a high risk of kidney failure, increased risk of loss of vision, hastening the progression of an increase in the risk of cardiovascular disease respectively[18-21]. The macrovascular complications include coronary heart disease, peripheral vascular disease, and stroke[22].

Conventional anti-diabetic therapy is not without the limitations of temporary therapeutic outcomes versus long-term cost implications. These limitations alongside the alarming increase in the prevalence of diabetes and the soaring cost of managing diabetes on a long-term basis are not commensurate with the low earning capacity of the affected populace, hence the need to encourage plant-based remedies.

The phytochemical analysis of the lime juice extract of the *Ficus exasperata* exhibited the presence of alkaloids, steroids, saponins, flavonoids, tannins, reducing sugar, and terpenoids which is responsible for many of its activities which include anti-oxidants and anti-inflammatory properties.

The *Ficus exasperata* plant *i*s widely used in African pharmacopoeia against hypertension, hemorrhoids, diabetes, skin diseases, and oedema[23]. The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated that combining insulin and oral hypoglycemic agents in patients with Type 2 Diabetes showed a reduced risk of complications and achievement of 'near-normal' glycemic control via the treat-to-target (TTT) algorithm[24.]

From our study, the group pretreated with insulin showed a steady decline in blood glucose level over the 120 min period. On the other hand, the pretreated group with the extract at 500mg/kg caused a significant reversal effect at 60- and 90-min indicative of insulin sensitivity. It is known from the literature that postprandial glucose level in normal subjects with optimal glucose tolerance is less than 7.8 mmol/L (140 mg/dL) in response to a meal and typically returns to premeal levels within two to three hours[25].

Findings from this study also showed in the glucose tolerance test of the LFE-pretreated group, that post-oral administration of glucose was associated with a significant reduction causing steady hypoglycemia over the succeeding 120 min. This reached significant hypoglycemic levels from 30 min at the dose of 500 mg/kg of the extract, arriving close to the pre-meal value at 120 min. Thus, the extract enhanced glucose utilization in glucose-loaded rats simulating what happens in a normal physiological state.

STZ is currently a long-established diabetogenic agent utilized in animal experimental protocols as a result of its effect on pancreatic β cells where it causes their destruction by selective necrosis[26]. In this present study, a single intraperitoneal injection of STZ caused hyperglycemia in the diabetic range (> 200 mg/dL) after 72 h, which lasted for 14 days (period of the course of the study).

Metformin is a first-choice oral hypoglycemic agent in the class of biguanides. It does not instantly reduce blood sugar levels. The effects are usually noticeable within 48 hours of taking the medication, and the most significant effects take 4–5 days to occur. This study also demonstrated that hyperglycemia was significantly attenuated in rats pretreated with metformin in addition to varying doses of LFE in a dose-related fashion, making the blood glucose fall within the range of basal value on days 7 and 14. Furthermore, there was no effect on blood glucose levels in non-diabetic rats as seen in the LFE-pretreated suggestive of normoglycemic action.

According to a study by Zafar and Naqvi[27], which demonstrated the effect of STZ-induced diabetes on the body weight of rats and the relative weights of the liver, pancreas, and kidney, there was a significant reduction noticed in the body weight of diabetic rats with an increase in the relative weights of kidney and liver and the weight of the pancreas was unaffected.

However, our study showed a statistically significant increase in the body weight of the LFE-pretreated group at the dose of 250 mg/kg ($p<0.01$) and 500 mg/kg ($p<0.0001$) in comparison to the STZ- treated group (which showed a progressive increase). The pattern of weight affectation in the LFE-pretreated-only group and the metformin-pretreated group were similar though not statistically significant. Furthermore, the body weights of the liver, pancreas, and kidneys in LFE-pretreated and STZ groups showed no statistical significance.

Several studies have shown that oxidative stress acts as a conjoint mechanism in the pathophysiology of diabetes

through the alteration in enzymatic systems, lipid peroxidation, impaired glutathione metabolism, and decreased vitamin C levels[28]. This is responsible for the initiation and progression of the disease. Defense mechanisms by the body to scavenge the deleterious effects of these free radicals are by the production of antioxidants (superoxide dismutase (SOD), glutathione peroxidase, and catalase) that will neutralize the elevated level of free radicals as seen in diabetes[29]. Natural phenolic compounds such as flavonoids contained in leaf extracts of *ficus exasperata* are known to possess strong antioxidant activity and free radical scavenging activities[30]. STZ caused significant deficit in antioxidant enzymes activity indicative of oxidative stress in pancreas and liver which were attenuated by LFE administration in a dose dependent manner, possibly due to presence of polyphenolic compounds. This is similar to a study by Feng *et al*[31], where mice were treated with flavonoids, which showed an upsurge in the activities of serum and liver superoxide dismutase (SOD), liver catalase (CAT), and blood and liver glutathione peroxidase (GSH-PX), however, a decline was demonstrated in the serum and liver malonaldehyde (MDA) content.

This has validated its alleged folkloric use in scientific studies as an anti-inflammatory agent for the treatment of oedema. It is also in tandem with a report by Abotsi *et al*[32] where the administration of *F. exasperata* leaf extract p.o reduced joint inflammation and prevented the systemic spread of immune- mediated arthritis. Ali Smith *et al* [33] evaluated the antioxidant activity of *F. exasperata* extract in four different assays by showing that mechanism of action of the extract as an anti-inflammatory agent was due to the total phenol content, reducing power effect, DPPH scavenging ability, and attenuation of lipid peroxidation.

Alteration in liver biomarkers: alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) are indicators of liver injury[34]. ALT (predominantly liver) and AST (heart, skeletal muscle, kidneys) in disproportion to ALP denote a hepatocellular disease whereas an elevation in ALP (bones, intestine, and placenta) in disproportion to ALT and AST would denote a cholestatic pattern[35].

A non-hepatotoxic pattern is implied based on the report from our study where the biochemical assay of ALT and ALP showed a significant increase in the STZ-treated group while at LFE 500 mg/kg (diabetic group) there was a statistically significant decrease. This decrease was also seen in LFE and MET treatment groups; LFE 50, 250 mg/kg and MET 500 mg/kg. A report demonstrated by Oyewole and Oladele[36] using the methanolic extract of *F. exasperata* at 500 mg/kg showed a decrease in the tissue level of ALT, ALP, and AST and an increase in serum level. This may suggest that the medicinal remedy of the extract is safe for use at lower doses and toxic at higher doses.

The electrolytes, urea, and creatinine are markers of kidney function[37]. Likewise, an elevated level of creatinine may indicate kidney disease. In this study, there was no significant increase in the levels of urea and creatinine which is suggestive that there was little or no damage to kidney function. Also, the LFE-treated group at 500 mg/kg showed no toxic effect on kidney function. This finding did not correspond to a study on the ethanolic extract of *ficus exasperata* at 500 mg/kg which was observed to have a deleterious effect on kidney function by increasing the level of urea and sodium and weight [38]. On a similar note, numerous studies have shown pathological and histological changes in the kidney following sub-chronic oral administration of an aqueous extract of *ficus exasperata* [39].

On the other hand, a study by Adewole *et al* [40] where the reno-protective property of *ficus exasperata* leaf aqueous extract was undertaken, and the report suggested that treatment with the extract ameliorates STZ-induced nephrotoxicity in the experimental diabetic study.

Streptozocin is one of the most common diabetogenic chemicals used in diabetes due to its specific necrosis of the pancreatic beta cells [41].

It is well noted that the weight of the pancreas for the treatment group (50, 250, and 500 mg/kg) and standard (metformin) group is reduced compared to the control group but is still slightly higher than the diabetic (STZ) group which may indicate that the extract is attempting to repair and restore the functions of the pancreas. Histoarchitectural damage of beta cells caused by drug-induced experiments in diabetic rats is regenerated by flavonoids which function as insulin secretagogues[42]. Reports have shown that aqueous leaf extract of *F. exasperata* is shown to cause functional beta cell regeneration of pancreatic islet cells.

Acute oral toxicity studies of the lime juice extract of *F. exasperata* did not produce any mortality or show any visible signs of toxicity after oral administration with a single dose of 5 g/kg p.o. Hence, the extract administered orally can be said to be relatively nontoxic since no death was observed up to the administration of 5 g/kg of the extract. Limitations to this study are stemmed from the fact that various fractions of *F. exasperata* exhibit different properties and characteristics, hence, further studies in the line of a comparative study of these various fractions will be

of great use in scientific research with regards to its application in medical remedy.

5.0 Conclusion

The phytochemical analysis exhibited the presence of substances such as flavonoids which could explain the antioxidant and anti-inflammatory effects. There was also a regeneration of the pancreatic islet cells which could be a potential therapeutic regime for treating Type 1 Diabetes mellitus. Although our findings from this study showed biochemical evidence of possible hepatic damage, in addition to anemia, and immunosuppression from the hematological parameters, many other positive effects such as kidney safety were demonstrated. Thus, with the antidiabetic activity demonstrated by the extract of *F. exasperata* in STZ-induced diabetes in rats similar to the use in folkloric use in diabetic treatment, it can be a potential therapeutic option though with caution and close monitoring.

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