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REVIEW ARTICLE

Anti-diabetic effects of *Ficus Asperifolia* in Streptozotocin-induced diabetic rats

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Abstract

Purpose The current study aimed to determine the antidiabetic effects of leaf extract of *Ficus asperifolia* in streptozotocin-induced diabetic rats.

Methods A total of ninety-five (95) adult rats were used for the experiment. The whole study protocol was divided into three sets of individual experiments. The animals were divided randomly into seven groups of five rats each. The rats were given a diet supplemented with 50 g high fat to 50 g vital feeds for two weeks. The study lasted 28 days with daily administration and weekly blood glucose and body weight check. At the end of the experiment protocol, the rats were fasted overnight and were anesthetized. Blood was collected via cardiac puncture from each animal for biochemical analysis. Metglim 3.38 mg/kg bodyweight was used as positive control. Diabetes was induced using streptozotocin (35 mg/kg in 0.1 M phosphate-buffered saline) intraperitoneally for 5 days. Phytochemicals were analyzed in both extract and fractions.

Results Phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, glycosides, tannins, carotenoids, terpenes, and steroids in both extract and fractions. Proteins, carbohydrates, and fats were detected by systematic molecular analysis. Fraction 1 of plant extracts prevented glucose-induced hyperglycaemia 30 min' post glucose load in all rats. Streptozotocin treatment caused a significant increase ($p<0.05$) in blood sugar, total cholesterol, triacylglycerol, low-density lipoproteins and a significant ($p<0.05$) decrease in food intake, body weight and high-density lipoproteins in diabetic rats.

Conclusion Treatment with the extract significantly improved the derangements caused by streptozotocin. These results suggest that the leaf extracts of *Ficus asperifolia* could serve as a potential treatment for diabetes therapy.

Keywords Diabetes · Oxidative stress · Plant extract · Phytochemical · Anti-oxidant · Drug candidate

Introduction

Diabetes Mellitus destroys pancreatic beta cells for decreased insulin production to up-regulate blood sugar concentration. Hyperglycemia in diabetes mellitus is a result of defects in the secretion, or resistance of insulin. Diabetes is one of the major causes of complications leading to worldwide death. Plant-based materials have been widely engaged lately in the quest for a permanent cure and research is still ongoing to find a safe, cost-effective, less side effect and readily available remedy for diabetes mellitus. Plant materials have a long history of containing effective molecules that mediate their anti-diabetic roles on the human body through processes identical to those already well understood for chemical compounds in conventional drugs, thus herbal medicines do not differ from conventional drugs in terms of how they work.

Many of these plants have been recognized for their several life-saving and therapeutic properties, and about 70% of the world population especially in the developing countries relies entirely on traditional therapies as their primary form of healthcare. In the recent years, secondary plant metabolites have been extensively investigated as source of medicinal agent [1, 2]. These secondary metabolites are different from plant to plant and examples include flavonoid, glycosides,

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saponins, tannins, alkaloid, sterols, quinones and anthraquinones. These active principles can be extracted and used in different forms including infusion, syrups, concoction, decoctions, infused-oils, essential oils, ornaments and creams. *Ficus asperifolia* (sandpaper tree) is a widely distributed plant across Africa and is readily available in Nigeria. It has been used as an analgesic, anti-cancer, diuretic, and as a menstrual pain reliever [1].

Conventional drugs used in the treatment of diabetes are sometimes inadequate, often expensive for the less-privileged people and can produce serious adverse effects including hypoglycemia, obesity, peripheral edema, and insulin resistance. Much research has been conducted to find more effective remedies for diabetes, however, despite such significant advances, diabetes is still a multifactorial, complex disorder with increasing incidence and prevalence. It is necessary to discover alternative drugs for the treatment of diabetes. We hypothesize that leaf extracts of *Ficus asperifolia* and their fractions may contain anti-diabetic molecules based on long-standing observation.

In this study, we determined the chemical constituents of *Ficus asperifolia* leaf extract as a potential drug candidate for diabetes through increased insulin sensitivity and anti-oxidant mechanisms. Alkaloids, saponins, and flavonoids were detected as functional molecules in high quantity from plant extracts. The proximate analysis of the leaf extract shows high carbohydrate content and a considerable amount of proteins.

The current study tested the hypothesis that *Ficus asperifolia* leaf extract could have anti-diabetic potentials using systematic, unbiased approach by series of biochemical analyses. Our specific aim is to determine the anti-diabetic and anti-lipidemic roles of *Ficus asperifolia* leaf extract on diabetic animals. Our experiments demonstrated that functional molecules from *Ficus asperifolia* could decrease blood sugar levels and lipid profile in diabetic animals. Importantly, drastic positive changes were observed in the food intake, body weight, and high-density lipoprotein (HDL-C) using plant extracts on diabetes. Our results suggest that *F. asperifolia* leaf extract have anti-diabetic and anti-hyperlipidemic molecules to treat diabetes as a new therapeutic application.

Materials and methods

Plant material

Matured leaf of *Ficus asperifolia* was collected from Song Local Government Area, Adamawa State, Nigeria. Song is located at latitude 9°049′28” N and longitude 12°037′30’E. The plant was identified and authenticated in the Department of Plant Science, Modibbo Adama University of Technology Yola-Adamawa State, Nigeria.

Animals

We followed the National Institutes of Health (NIH) Guide and the Association for Research in Vision and Ophthalmology (ARVO) statement for in vivo experiments. Ninety-five (95) male Wistar strain rats (7 weeks, 100 ± 20 g) were obtained from the Animal House Unit, National Veterinary Research Institute Vom, Plateau, Nigeria. Animals were housed in polypropylene cages and were given Broiler finisher (vital feeds) diet and water ad-libitum. Animals were maintained under laboratory conditions of 25 °C temperature and 12 h light and dark cycle before the experiments.

Metglim as positive control

Metglim was obtained from Hovid Pharmaceuticals, UK and was used as the reference drug as anti-diabetic medicine.

Equipment

Spectrophotometer (Model-1371, Ryan Science and Instrument Company, England) was used for plant extract analysis. Furnace, dry air oven (Gallenhamp oven 300 series plus), distillation apparatus, glucometer, and column chromatography (Bulk the scientific USA) were used for temperature control, component analysis, glucose analysis, and fractionation, respectively.

Chemicals

Streptozotocin (STZ) was purchased from Sigma (St Louis, MO, USA). Kits for lipid profile analysis were purchased from Randox Lab (UK). All chemicals were analytical grade.

Ficus asperifolia leaf

The leaf was washed, dried in the dark and was ground to powder using a pestle and mortar. The leaf powder (260 g) was extracted with ethanol (750 ml × 3) in an airtight flat bottom container for 48 h with occasional shaking until the major extractable component of the plant was fully dissolved in the solvent. The mixture was filtered using a muslin cloth and the filtrate was concentrated at 45 °C using a rotary evaporator. The remaining solvent was evaporated in a vacuum oven at 45 °C to obtain the crude plant extract and further fractionated using column chromatography.

Column-chromatography

A cylinder-shaped glass column containing stationary phase (silica gel) is poured slowly from the top with liquid solvents mixture dichloromethane, n-hexane, and methanol in the ratio of 10:10:1. The mobile phase flows down the column with the

gravity or external pressure. This technique was used for the purification of compounds from a mixture. The compounds in the mixture have different interactions ability with the stationary phase (silica gel) and mobile phase, thereby flowing along with the mobile phase at different time intervals. The individual compounds are collected as fractions and analyzed further for structural elucidation.

Isolation and purification of bioactive compounds from plant samples

A suitable sized long cylindrical glass column (based on the amount of the sample) was set up on a column-chromatography stand. A completely dried plant extract sample was mixed with silica gel to make a fine powdered form for easy distribution of the sample in the already packed silica gel column. Sample powdered mass was placed on the top of the pre-packed silica column and the sample was covered with a layer of cotton. Then, solvents of different polarities were passed through the column at a uniform rate under gravity to fractionate the sample extract. Each fraction was collected separately in a test tube and numbered consecutively for further analysis for thin-layer chromatography. Thin-layer chromatography (TLC) provided partial separation of both organic and inorganic materials using thin-layered chromatographic plates especially useful for checking the purity of fractions. Each fraction was applied on activated TLC plates with the help of capillary tube at a 1/2 in. apart from the lower edge of TLC plate, and the plate was kept in a developing chamber containing suitable solvent system for the specific time until the developing solvent reaches the top of the upper edge of TLC plate. The plate was taken out from the developing chamber, dried and the solvent front is marked by lead pencil. Compound spots on TLC chromatophores were detected by visual detection under UV light (254 nm), in iodine chamber or by using spray reagent (vanillin-sulphuric acid) for the presence of specific compounds. The visualized spots of the components in the chromatophores were marked and the Rf value of each spot was calculated by the formula as Rf = distance travelled by the sample (cm)/distance travelled by the solvent (cm). TLC plate showing the number of spots (compounds) for each fraction was further purified using high-performance liquid chromatography (HPLC). Based on the nature of the compounds, further spectral analyses including infrared (IR), mass spectrometry, and nuclear magnetic resonance (NMR) were performed to elucidate the chemical structure of target compounds.

Determination of chemical constituents

Phytochemical screening

Phytochemical analysis was carried out on the *Ficus asperifolia* ethanol leaf extract to identify the constituents using standard procedures as previously reported by Sofowora et al. [3] and Evans et al. [4].

Proximate analyses

Moisture Moisture content was measured using air-oven following official methods of the Association of Official Analytical Chemists [5]. A material test chamber M720 (Binder GmbH, Tuttlingen, Germany) was used to dry the samples until constant weight. The percentage of moisture content was calculated as:

$$\%Moisture = 1 - Weight\ dry\frac{sample}{Weight}wet \text{ sample x } 100$$

Lipid The determination of lipid content was performed following a Soxtec method previously described by Noureddini et al. [6] using a Soxtec TM 2050 automated analyzer (FOSS Analytical, Hillerød, Denmark). Petroleum ether was used for the extraction, whereas the percentage of lipid was obtained following equation below:

$$\%Lipids = Weight\ extraction\ cup + residue - Weight\frac{extraction\ cup}{Weight}sample\ x\ 100$$

Protein The Kjeldahl method was performed according to method 981.10 of the AOAC International [7]. Approximately 1 g of raw material was hydrolyzed with 15 mL concentrated sulfuric acid (H_2SO_4) containing two copper catalyst tablets in a heat block (Kjeltec system 2020 digestor, Tecator Inc., Herndon, VA, USA) at 420 °C for 2 h. After cooling, H_2O was added to the hydrolysates before neutralization and titration. The amount of total nitrogen in the raw materials were multiplied with both the traditional conversion factor of 6.25 [8].

Ash content and ash solution A dry ashing method was used to determine the ash content [9, 10]. The samples were incinerated in a furnace (Furnace 62,700, Barnstead/Thermolyne, Dubuque, IA, USA) at 550 °C. The remaining inorganic material was cooled, weighed and further used for the determination of mineral contents. An ash solution was prepared by dissolving the ash in 100 mL of 1 M HCl.

Carbohydrate and caloric value The total carbohydrate content (%) in the samples was calculated by the difference method. The caloric value was calculated by the sum of the percentages of proteins and carbohydrates multiplied by a factor of 4 (kcal/g) and total lipids multiplied by a factor of 9 (kcal/g).

In vivo experiments

A total of ninety-five rats were used (55 diabetic and 40 control rats) with five (5) rats in each group. The study was divided into three sets: Experiments 1 and 2 were performed for the Oral Glucose Tolerance Test (OGTT) of *Ficus asperifolia* fractions on 30 diabetics and 30 control rats, respectively. Metglim, fraction 1, 2 and 3, crude extract (200 mg/kg body weight) were administered to diabetic rats as well as the normal rats after 30 min of glucose (2 g) administration. Blood glucose concentration from tail vein was measured by using Glucometer at 0, 30, 60, 90, and 120 min, for both diabetic and normal rats. The 3rd experiment was designed for long-term treatment. Thirty-five male albino rats (7 weeks, 100 ± 20 g) were randomized into seven (7) groups of five (5) rats each. The animals were divided into different groups. Group I: nondiabetic animals received only broiler finisher feed with water. Group II: nondiabetic animals were administered 400 mg/kg of ethanol leaf extract. Animals in Group III-VIII was diabetic. Group III was given broiler finisher feeds and water and considered as a negative control (diabetic control). Group IV was given Metglim 100 mg/kg orally once daily as a positive control. Group V, VI and VII animals received 100, 200 and 400 mg/kg of the extract, respectively. The experiment lasted for 28 days.

Induction of diabetes

Types 2 diabetes was induced as previously reported by Srinivasan et al., [9]. The rat's diet was supplemented with 50 g high fat to 50 g vital feeds for two weeks. The overnight fasted animals weighing between 200 and 250 g was injected 35 mg/kg streptozotocin (dissolved in 0.1 M normal saline) intraperitoneally for five (5) days. After four days, blood glucose was examined using a blood glucose meter (Accu-Chek Performa; Roche Diagnostics, USA). Rats with a blood glucose of 300 mg/dl and above were considered diabetic and were used for experiments. The blood glucose concentrations and body weights were measured every week and the feed intake daily was taken in the course of the treatment.

Experimental design

To test the hypothesis, we administered streptozotocin in experimental rats fed with high-fat diet for two weeks. Next, the sugar level was analyzed after a week and the rats with most elevated sugar levels were considered diabetic was treated with different concentrations of leaf extract of *Ficus asperifolia* for four weeks. The body weight and food intake were analyzed weekly. At the end of the experiment, the animals were euthanized and the blood sample was collected for sugar concentration and lipid profile test. Fractions separated by column chromatography were administered to diabetic and normal rats to examine the OGTT.

Standard drug administration

Metglim 3.38 mg/kg body weight was administered orally as a reference control drug.

Statistical analysis

Data are expressed as mean of 5 replicates ± standard deviation. The obtained data were subjected to statistical analysis using the IBM® Statistical Package for Social Sciences (SPSS) Software Version 23. All significant differences were determined by one-way Analysis of Variance (ANOVA) and Post Hoc multiple comparisons were done using Duncan's multiple range test. The significance level was set at $p < 0.05$.

Results

Qualitative phytochemicals in *Ficus asperifolia* leaf extract and fractions

The result of the preliminary phytochemical screening of *Ficus asperifolia* leaf extract and fractions are shown in Table 1 with flavonoids, saponins, alkaloids, glycoside, steroids among others are present while fractions of *F. asperifolia* leaf extract by ethanol shows the absence of three components. Saponins, glycosides, and steroids were present in all the fractions. Fraction 2 (F2) has alkaloids and carotenoids, where as it is not present in other fractions F1 and F3. Flavonoids were only present in fraction 1.

Preliminary phytochemical screening of the crude extract of *Ficus asperifolia* revealed the presence of flavonoids, saponins, glycosides, alkaloids, steroids, tannins, carotenoids and terpenes among other secondary metabolites (Table 1).

Table 1 Qualitative analysis of phytochemicals in Ficus asperifolia leaf extract and fractions

Phytochemical	Crude Extract	Fraction 1	Fraction 2	Fraction 3
Flavonoids	+	+	–	–
Saponins	+	+	+	+
Glycosides	+	+	+	+
Alkaloids	+	–	+	–
Steroids	+	+	+	+
Tannins	+	–	–	–
Carotenoids	+	–	+	–
Terpenes	+	–	–	–

Quantitative analysis of phytochemicals in *Ficus asperifolia* leaf extract and fractions

Table 2 shows the levels of specific phytochemicals present in *Ficus asperifolia* leaf extract and fractions. Alkaloids, saponins, and flavonoids are present in substantial amounts in the extract while carotenoids, steroids, and terpenes are in minimal concentration in the extract. Fraction 2 has the lowest level of steroids and is the only fraction with the presence of alkaloids. Table 1 and Table 2 show the results of qualitative and quantitative screening of ethanol extract and fractions of *Ficus asperifolia*. High amounts of alkaloids, saponins, flavonoids, and glycosides in both the crude extract and fractions may explain the observed biochemical effects.

Proximate content of *Ficus asperifolia* leaf extract

The result of the proximate content of *Ficus asperifolia* leaf extract is shown in Table 3. Protein, carbohydrate, and ash were present in a substantial amount in the extract. Table 3 present the proximate chemical composition of the *Ficus asperifolia* leaf sample. The sample contains a considerable amount of carbohydrate $(C_6H_{12}O_6)_n$, fat, crude fibre, ash and moisture from leaf extract. The proximate composition in *Ficus asperifolia* leaf shows carbohydrate (CHO), protein, moisture, ash, crude fibre in high concentrations of potential dietary value. These results are in correlation with the findings of Aja et al. [10], except for protein with high variation. This variation could be a result of a difference in climate, time of the year and different soil type where the sample was collected.

High levels of proteins (19.97 ± 2.01%) as presented in Table 3 suggest that *Ficus asperifolia* leaf could be important source of amino acids and components of biological membranes. Carbohydrate (35.34 ± 2,81%) may serve as a source of energy, whereas crude fiber (5.17 ± 0.01%) may help in digestion. Moisture (9.95 ± 0.63%) is the resource of blood and oxygen transport. Ash (9.30 ± 0.62%) represents mineral content in food which is required by the body for proper physiological functioning. The result of this study indicated that *Ficus asperifolia* is a good source of the nutrient.

Table 2 Quantitative analysis of phytochemicals in Ficus asperifolia leaf extracts and fractions in mg/g

Phytochemical	Crude Extract	Fraction 1	Fraction 2	Fraction 3
Flavonoids	31.47 ± 3.03	0.60 ± 0.03	–	–
Saponins	41.77 ± 4.03	0.80 ± 0.05	3.56 ± 0.02	1.90 ± 0.41
Glycosides	16.70 ± 2.00	1.00 ± 0.21	3.60 ± 2.00	2.10 ± 1.00
Alkaloids	58.60 ± 6.06	–	10.60 ± 0.01	–
Steroids	6.37 ± 1.03	1.60 ± 0.81	1.55 ± 0.71	3.00 ± 1.99
Tannins	9.67 ± 1.03	–	–	–
Carotenoids	4.90 ± 0.96	–	7.75 ± 5.00	–
Terpenes	7.17 ± 1.09	–	–	–

Values are expressed as Mean ± S.E.M

Table 3 Proximate content of Ficus asperifolia leaf extract

Proximate Content	Amount (%)
Crude Protein	19.97 ± 2.01
Fat	8.25 ± 0.50
Crude Fibre	5.17 ± 0.01
Ash	9.30 ± 0.62
Moisture	9.95 ± 0.63
Carbohydrate	35.34 ± 2.81

Values are expressed as Mean ± S.E.M

Effects of *Ficus asperifolia* extract on food intake of high fat/streptozotocin-induced diabetic rats

A result of food intake is presented in Table 4. Week one represents the first week after treatment with *Ficus asperifolia* leaf extract and standard drug whereas week four is the fourth and last week of treatment before animals were euthanized. There was 20.10% increase intake in normal rats as compared to −17.36% decrease in food intake of diabetic rats. Food intake of the diabetic group was high before treatment with plant extract commenced. The results show significant decrease in food intake as the disease progresses.

Effects of *Ficus asperifolia* extract on body weight of high fat/ streptozotocin-induced diabetic rats

The effects of *Ficus asperifolia* leaf extract on body weight for four weeks after induction is shown in Table 5. There is a −16.29% decrease in body weight of diabetic control rats as compared to a 25.50% increase in normal control. Treatment with the Metglim and the extract (100, 200 and 400 mg/kg/b. w) has significantly ($p < 0.05$) increased the body weight by 20.54%, 5.56%, 12.5% and 19%, respectively. Bodyweight was recorded every week and the final data are shown in Table 5. There was a significant decrease in body weights in diabetes rats (−16.29%) compared to non-diabetic animals (25.50) ($p<0.005$). The ethanol extracts of *Ficus asperifolia* (400 mg/kg/b. w) caused a significant increase in weight in diabetic rats ($p<0.005$).

Effects of *Ficus asperifolia* fraction on OGTT normal rats

Figure 1 shows the effect of each fractions, Metglim and crude extract in normal rats. There is no significant difference

Table 4 Effects of Ficus asperifolia on food intake of high fat /streptozotocin-induced diabetic rats

Food Intake	Baseline	Week 1	Week 4	% change F.I
Normal control	75.25 ± 1.00[b]	72.44 ± 3.99[b]	87.00 ± 5.02[b]	20.10
Ethanol Extract 400 mg/kg b. w	73.10 ± 002[b]	66.89 ± 3.27	80.71 ± 4.88[b]	20.66
Diabetic control	60.55 ± 005	54.20 ± 1.36[a]	44.79 ± 1.08[a]	−17.36
3.38 mg/kg b. w Metglim	61.25 ± 0.20	65.58 ± 3.19	70.33 ± 3.89[b]	7.24
Diabetic +100 mg/kg b. w EE	63.10 ± 1.35	54.37 ± 1.50	60.72 ± 2.99	11.68
Diabetic +200 mg/kg b. w EE	60.85 ± 1.80	63.36 ± 3.02	69.27 ± 3.30	9.33
Diabetic +400 mg/kg b. w EE	62.80 ± 0.50	70.55 ± 3.76[b]	77.92 ± 4.18[b]	10.45

Values are expressed as Mean ± SEM $n = 5$

[a] = significantly ($p < 0.05$) lower compared to normal;

[b] = significantly ($p < 0.05$) higher compared to diabetic control;

EE = ethanol leaf extract; b.w = body weight, F.I = food intake

between rats administered Metglim, fractions 1 and 3 and normal rats two hours after a glucose load.

Effects of *Ficus asperifolia* fraction on OGTT of streptozotocin-induced diabetic rats

Figure 2 shows the effects of the fractions, standard drug and crude extract on diabetic rats. Fraction 3 shows a significant ($p < 0.05$) decrease in the blood glucose of diabetic rats 90 min after glucose load which continues to decline until 120 min. There is no significant ($p < 0.05$) difference between fraction 3 and Metglim treated rats. The effect of *Ficus asperifolia* on glucose tolerance is presented in Fig. 2 as a dose-dependent manner. In glucose-fed animals treated with distilled water, there was a significant increase in blood glucose levels after 30 to 90 min following the administration of glucose ($P < 0.05$). The maximum increase in blood glucose was observed 30 min after the administration of glucose. The *Ficus asperifolia* fraction 1–3 (200 mg/kg) and Metglim (10 mg/kg) significantly prevented a rise of the blood glucose level ($P < 0.05$) after 30 and 120 min compared to the control group.

Effects of *Ficus asperifolia* extract on blood glucose in high fat/ streptozotocin-induced diabetic rats

Glucose levels of rats fed a high fat and multiple doses of streptozotocin raised to as high as 368.00 ± 15.08 mg/dL as shown in Fig. 3 (Supplement Table C) which continues to decline significantly $p < 0.05$ in the diabetic control groups even after four weeks of treatment. Treatment with Metglim shows a significant decrease (154.75 ± 6.55) in blood glucose levels compared to diabetic control (376.40 ± 10.91). Treatment with extract at a higher dose (400 mg/kg) shows significant ($p < 0.05$) reduction in blood glucose levels compared to diabetic control. Streptozotocin-induced diabetes resulted in a significant increase in serum glucose level (368.00 ± 15.08, 364.25 ± 15.43, 366.85 ± 12.66, 369.75 ± 11.03,

Table 5 Effects of Ficus asperifolia on body weight of high fat/streptozotocin-induced diabetic rats

Treatment	Baseline	Week 1	Week 4	% change b.w
Normal control	177.63 ± 6.37	186.68 ± 6.50	234.22 ± 5.84	25.50
Ethanol Extract 400 mg/kg b.w	186.86 ± 5.49	196.54 ± 5.51	251.92 ± 4.89[b]	28.20
Diabetic control	230.68 ± 5.75	223.06 ± 5.86	186.73 ± 4.90[c]	−16.29
3.38 mg/kg b.w Metglim	228.77 ± 6.43	230.03 ± 6.53[a]	277.28 ± 6.72[bd]	20.54
Diabetic +100 mg/kg b.w EE	230.16 ± 4.41	232.96 ± 8.56[a]	245.91 ± 3.88	5.56
Diabetic +200 mg/kg b.w EE	230.16 ± 7.22	234.75 ± 3.99[a]	262.32 ± 3.86[b]	12.00
Diabetic +400 mg/kg b.w EE	229.31 ± 4.00	236.71 ± 4.99[a]	281.02 ± 4.63[bd]	19.00

Values are expressed as Mean ± SEM n = 5

[a] = significantly ($p < 0.05$) higher compared to normal in week 1

[b] = significantly ($p < 0.05$) higher compared to diabetic control in week 4

[c] = significantly ($p < 0.05$) lower compared to normal and treatment group in week 4

[d] = significantly ($p < 0.05$) higher compared to treatment with 100 & 200 mg/kg/b.w in week 4

EE = ethanol leaf extract; b.w = body weight

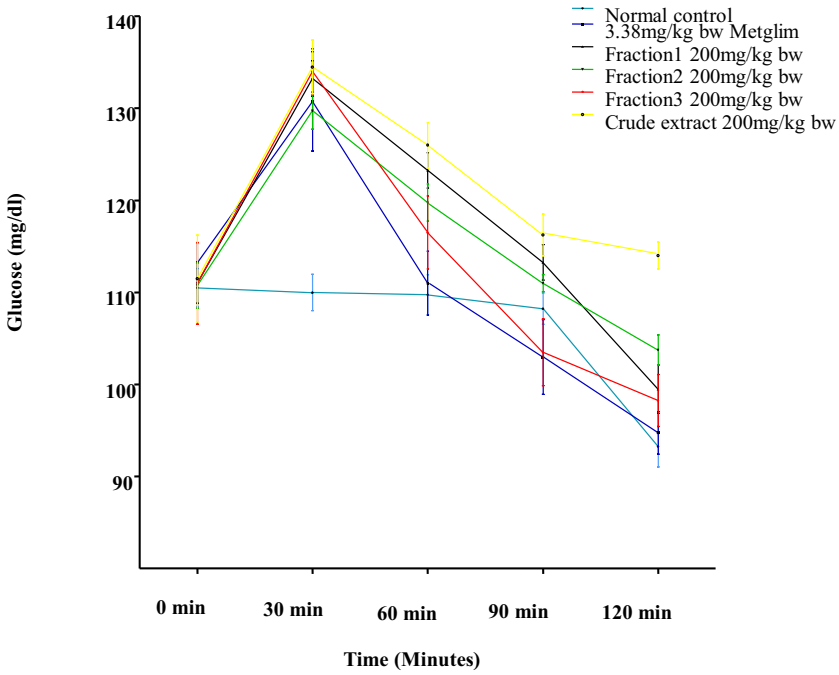

Fig. 1 Effects of fractions and crude extract of *Ficus asperifolia* on Oral Glucose Tolerance Test (OGTT) on normal rats. Administration of these fractions (I, II, III) at different concentrations and crude extracts at 200 mg/kg/b. w show a significant decrease as compared to normal untreated rats

376.40 ± 10.91) in comparison to the normal control group (96.75 ± 2.78, 88.00 ± 3.14, 81.00 ± 2.16, 83.00 ± 2.16, 80.25 ± 0.75) ($p<0.005$). After the administration of the different doses of *Ficus asperifolia* extract (100, 200 and 400 mg/kg) to diabetic rats during 28 days a significant decrease in blood glucose levels ($p<0.005$) was observed. Supplement Table C and Fig. 3 shows the effect of *Ficus asperifolia* extract on blood glucose concentration of diabetic albino rats. All the diabetic animals treated with various doses of the extract showed a significant decrease in blood glucose concentration ($p<0.005$) when compared to the diabetic control rats.

Effects of *Ficus asperifolia* extract on lipid profile on high fat/ streptozotocin-induced diabetes type 2 rats

Table 6 shows the effects of *Ficus asperifolia* extract on specific lipid profile parameters, including total cholesterol (TC,230.05

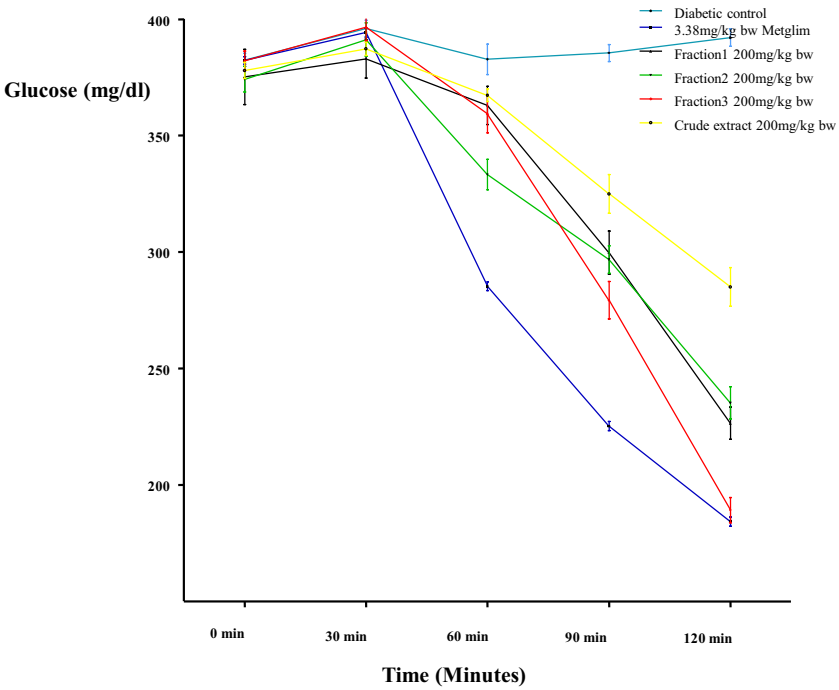

Fig. 2 Effects of fractions and crude extract of *Ficus asperifolia* on Oral Glucose Tolerance Test (OGTT) on diabetic rats. Fractions of *Ficus asperifolia* administered to diabetic rats show a significant ($p<0.005$) decreased in the oral glucose tolerance test (OGTT) at 120 min after glucose load especially at F3 and Metglim treatment as compared to diabetic control

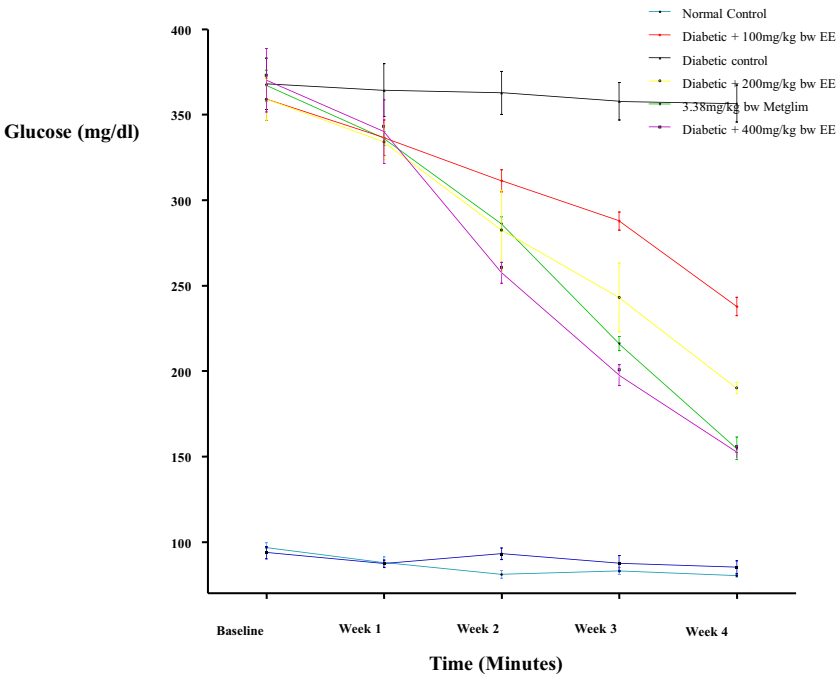

Fig. 3 Effects of extract of *Ficus asperifolia* on fasting blood glucose on high-fat/ streptozotocin-diabetic rats. Administration of ethanol leaf extracts of *Ficus asperifolia* on fasting glucose in diabetic rats shows a significant decrease especially at higher doses and at week 4 as compared to normal control

± 0.55 mg/dL), high density lipoprotein (HDL, 20.45 ± 1.19 mg/dL), low density lipoprotein (LDL,143.73 ± 2.32 mg/dL) and triacylglycerol (TAG, 213.00 ± 0.91 mg/dL) in diabetic control as compared to the normal rats TC (185.75 ± 0.85 mg/dL), HDL (47.25 ± 0.48 mg/dL), LDL (99.00 ± 0.41 mg/dL) and TAG (131.25 ± 2.72 mg/dL), respectively. Serum cholesterol levels in diabetic rats showed a significant increase in comparison to non-diabetic control rats (230.05 ± 0.55 mg/dL) vs (185.75 ± 0.85 mg/dL). Serum triacylglyceride levels were also elevated in diabetic rats (213.00 ± 0.91 mg/dL) compared to non-diabetic animals (131.25 ± 2.72 mg/dL) ($p<0.005$). The obtained results were significantly different from the normal control animals. The effects of the extract on the lipid profile parameters of diabetic rats are presented in Table 6. All the treated animals showed a significant decrease ($p<0.005$) in the concentration of serum TAG, TC and LDL and a significant increase ($p<0.005$) in serum HDL concentration when compared to the diabetic control animals. Table 6 shows the effect of the extract on serum LDL and HDL concentrations. There was a significant reduction ($p<0.005$) of low-density lipoproteins-cholesterol concentrations in the animals treated with the various doses of the extract.

Discussion

Phytochemistry focuses on varieties of secondary plant metabolites. These plants represent a treasure trove of structurally

Table 6 Effects of Ficus asperifolia on lipid profile on high fat/ streptozotocin-induced diabetes type 2 rats (mg/dl)

Treatments	TC	HDL	LDL	TG
Normal Control	185.75 ± 0.85[a]	47.25 ± 0.48[b]	99.00 ± 0.41[c]	131.25 ± 2.72[c]
Normal + Ethanol Extract 400 mg/kg/b. w	174.25 ± 2.02[a]	41.75 ± 0.85[b]	103.00 ± 2.48[c]	122.25 ± 2.25[c]
Diabetic Control	230.05 ± 0.55	20.45 ± 1.19	143.73 ± 2.32	213.00 ± 0.91
Diabetic rats +3.38 mg/kg/b. w Metglim	191.75 ± 2.25[a]	39.25 ± 0.82	111.75 ± 2.25[c]	140.50 ± 0.65[c]
Diabetic rats + EE 100 mg/kg/b. w	213.75 ± 1.44	29.00 ± 1.29	139.50 ± 0.65	210.75 ± 1.25
Diabetic rats + EE 200 mg/kg/b. W	204.50 ± 0.65	33.00 ± 0.41	133.75 ± 2.39	167.50 ± 0.65
Diabetic rats + EE 400 mg/kg/b. w	193.25 ± 1.25[a]	40.00 ± 0.63[b]	113.00 ± 0.41[c]	147.50 ± 0.65[c]

Values are expressed as Mean ± SEM n = 5

[a] = significantly ($p < 0.05$) lower compared to diabetic control in TC column

[b] = significantly ($p < 0.05$) higher compared to diabetic control in HDL column

[c] = significantly ($p < 0.05$) lower compared to diabetic control in LDL & TG column

EE = Ethanol Extract, TC = Total Cholesterol, HDL = High density lipoprotein, LDL = Low density lipoprotein and TG = Triacylglyceride

diverse bioactive molecules. The physiological and therapeutic effects of plant materials typically result from the combinations of these secondary products in plants [11]. The information on the constituents of the plant clarifies the uses of the plants but only a small percentage have been investigated for their phytochemicals and only a fraction has undergone biological or pharmacological screening. This probably explains the earlier reported medicinal use of *Ficus asperifolia* by Omoniwa et al. [12]. Our previous study also reveals the vital role of the presence of phytochemicals in plant extracts [13].

Alkaloids are heterocyclic nitrogenous compounds. They act as antihyperglycemic agents by inhibiting the activity of disaccharides in colorectal adenocarcinoma (Caco-2) cells. It decreases sucrase activity after pre-incubation with Caco-2 cells suggesting that the antihyperglycemic activity of alkaloid is due to its ability to inhibit alpha-glucosidase and decrease glucose transport through intestinal epithelium [14]. Alkaloids also have the potential for diabetic treatment because they reduce diet-induced hyperglycemia and endogenous insulin-secretion by inhibiting intestinal R-glucosidase [15, 16]. Alkaloids have also been reported to have analgesic and anti-inflammatory activities which help to alleviate pains, develop resistance against disease and endurance against stress.

Saponins are steroids and triterpene, glycosides. They are called saponins due to their soapy-like properties. Saponins possess anti-ulcer, anti-tumor and anti-diabetic properties [17]. Saxena et al. [18] reported membrane-permeabilizing, Immuno-stimulant, hypocholesterolaemia and anti-carcinogenic properties of saponins and they have also been found to significantly impair the digestion of protein and the uptake of vitamins and minerals in the gut to cause hypoglycemia. Saponins are bioactive against diabetes they stimulate secretion and regeneration of β-cells islet and activate the enzymes which are responsible for glucose utilization [19, 20].

Flavonoids may inhibit vascular diseases' development through alteration in endothelial cell eicosanoid production. Flavonoids may have a beneficial action in obesity due to their capacity to regulate fatty oxidation and improve adipocyte functionality. Flavonoids have been reported to exhibit various biological functions and medicinal properties such as antioxidant, anti-inflammatory, and antidiabetic. It has been proven to significantly lower the risk of atherosclerosis and cardiovascular diseases (CVD) as reported by Salvamani et al. [21]. Flavonoids have been reported to modulate carbohydrate and lipid metabolism, attenuate hyperglycemia, dyslipidemia and insulin resistance, improve adipose tissue metabolism, and alleviate oxidative stress and stress-sensitive signalling pathways and inflammatory processes [22–24].

Tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, hemicellulose, pectin, etc.), nucleic acids and minerals [25]. Tannins are potential antioxidants. They have been considered to be cardio-protective, anti-inflammatory, anti-carcinogenic and anti-mutagenic, among others. Tannins enhance glucose uptake and inhibit adipogenesis, thus being potential drugs for the treatment of NIDDM. Tannins can improve the pathological oxidative state of a diabetic situation [26].

Proximate composition of the *Ficus asperifolia* extract analyzed were crude protein, fats, fiber, ash, moisture and carbohydrate (Table 3). The proximate composition determines the nutritional quality of the extract [27]. The high level of crude protein in the extract indicates that it could be a good source of affordable dietary amino acids. Proteins are essential for the biosynthesis of vital structural components including antibodies, hormones and muscles [28]. Crude fiber has been reported by Weickert et al. [29] to modulate diabetes by reducing appetite and enhancing weight loss. Post et al. [30] reported that food rich in fiber are good modulators of post-prandial hyperglycemia. High insoluble fiber diets have also been reported to reduce diabetes risks by enhancing insulin sensitivity [31]. The dietary fiber content of the plant extract could be responsible for their use in the control of diabetes.

A significant drop in food intake in diabetic rats was observed in this study (Table 4). This could be due to appetite loss caused by gastroparesis which occurs because the nerves that move food through the digestive tract are damaged, so muscles don't work properly. As a result, foods remained undigested in the stomach. The treatment has shown a significant ($p < 0.05$) increased in the food intake as shown in the Table 4 which may be due to the presence of vitamins and minerals present in the extract which may have caused food digestion thereby, restoring the appetite. In addition, we expected that severe and mild diabetic condition may show different trends of food intake. For example, mild diabetic condition may increase food intake whereas severe diabetic condition may decrease food intake. For dose-dependent manner of leaf extract administration, we assumed the threshold effect beyond optimum level or allosteric inhibitory effect of the extract.

A significant ($p < 0.05$) decrease in body weight was observed in diabetic rats as shown in Table 5. This is widely associated with diabetic patients due to loss of appetite which was also confirmed in the study by the decline in food intake in Table 4. Table 5 showed an increase in the bodyweight of animals treated with the extract. Aja et al. [10] reported a significant ($p < 0.05$) reduction in the mean body weight of diabetic control compared to the positive group while rats in the treated groups showed significant ($p < 0.05$) increased in their mean body weight compared to the diabetic control group. Grover et al. [32] had also reported that aqueous extract of Aeglemarmelose leaves was equally effective in comparison to insulin in restoring blood glucose and body weight to normal levels.

When extract fractions of *Ficus asperifolia* were administered to diabetic and normal rats (Figs. 1 and 2), a significant ($p < 0.05$) decrease was observed in the oral glucose tolerance test (OGTT) 2 h after glucose load. Diabetes mellitus of long duration is associated with several complications including atherosclerosis, myocardial infarction, and neuropathy. These complications have long been assumed to be related to chronically elevated blood glucose levels. Diabetes mellitus causes disturbances in the uptake of glucose as well as glucose metabolism. The experiment showed that Glucose Tolerance Test (GTT) measures the body ability to use glucose, the body's main source of energy [33]. This test can be used to diagnose pre-diabetes and diabetes. Glucose lowering effects were found after oral administration of fractions and crude extracts in rats. The effect could be attributed to the hypoglycaemic effects of flavonoids, saponins, alkaloids, glycosides, and steroids present in a reasonable amount in the fractions. This result agrees with the earlier report by Nadro et al. [34] who suggested that fractionation helped in freeing up the hypoglycaemic agents in the fractions which confer protective effect as the standard drug. The result is also consistent with the previous study by Mohammad et al. [35] who reported that active fraction of *Heliotropium indicum* by methanol has enhanced the utilization, so blood glucose levels were significantly decreased in glucose loaded rats.

Intraperitoneal administration of streptozotocin led to the elevation of blood glucose levels in the diabetic untreated groups (Fig. 3). This result is consistent with several studies in rats. Administration of *Ficus asperifolia* extract to diabetic rats, however, caused a significant ($p < 0.05$) decrease in blood glucose concentration as compared to diabetic control. The highest dose of the extract (400 mg/kg body weight) was in synergy with the Metglim (standard diabetic drug) treated groups. A reasonable amount of saponins may be responsible for the hypoglycaemic effect of the extract as reported by Abdel-Zaher et al. [19], to stimulate regeneration of β-cells islet and activates the enzyme responsible for glucose utilization. This is in accordance with the previous report by Gupta et al. [36] and Tapas et al. [37] who reported that some bioactive compound may be responsible for the observed hypoglycaemic effect. This result is in agreement with Nadro et al. [38] report on the reduction in blood glucose as a result of plant material administration.

There was a significant increase in total cholesterol (TC), low-density lipoprotein (LDL), Triglyceride (TG) levels and decreased high-density lipoprotein (HDL) of streptozotocin-induced diabetic rats (Table 6). This is an indication of damage caused by administration streptozotocin. Treatment with *Ficus asperifolia* ethanol leaf extracts significantly ($p < 0.05$) reduced the levels of TC, LDL, and TG levels in diabetic treated rats as compared to diabetic control rats. While the increased level of HDL was observed in the diabetic group treated with the extract compared to diabetic control rats. In diabetic control has 20 mg of HDL whereas diabetic rat treated with Metglim shows 39 mg, meaning that increased HDL is the result of drug treatment. Higher concentration of leaf extract (400 mg) increased HDL (40 mg) closed to positive control (Metglim treated). Our results demonstrate that 400 mg of leaf extract is efficient as Metglim in HDL regulation. Flavonoids in the extract may be responsible for the beneficial effect. Salvamani et al. [21] reported flavonoids to lower the risk of atherosclerosis and cardiovascular disease. This result is in agreement with the study of Patrick et al. [39] and Yadav et al. [40] on the hypolipidemic effects of plant extracts. The result is also consistent with the findings of Yusufoglu et al. [41] that oral administration of *Ficus duranii* reversed the changes in plasma lipoprotein of diabetic rats and significantly improved their values towards normalcy [42–44].

Extracted molecules from *Ficus asperifolia* may interfere insulin signaling as insulin-insulin receptor-insulin receptor substrate-PI3K-AKT-GS3K-Glut4 as tyrosine kinase pathway. We suspect that a specific molecule of plant extract may bind to one of the receptor proteins to inhibit diabetic pathway. Currently we try to simulate the binding assay between extracted molecules and diabetic pathway proteins.

Conclusion

Treatment with the *Ficus asperifolia* extract significantly improved the derangements caused by streptozotocin-induced diabetes in vivo using animal model. It can be concluded from the study that components of the leaf, including flavonoids, alkaloids, saponins and other bioactive components that are known to have pharmacological benefits could be responsible for the observed effects. These results suggest that extracts of *Ficus asperifolia* could serve as a potential medicine for diabetes therapy. Further studies need to be carried out to define the mechanism of bioactive molecules in *Ficus asperifolia.*

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Compliance with ethical standards

Conflict of interest There is no conflict of interest.

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