



Antidiabetic and Antioxidant Activities of Extract of *Entandrophragma Cylindricum* (Sprague) Leaves in Male Wistar Rats

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Abstract

Background & Aims: *Entandrophragma cylindricum* (EC) is a tree with a widespread presence in various West African countries. It has wide folkloric use as an anti-sickling, antimalarial, analgesic, anti-inflammatory, and is widely used traditionally in treating diabetes across West Africa. The purpose of this research is to evaluate the antidiabetic potential of the methanolic leaf extract of *Entandrophragma cylindricum* (EC) in rats.

Materials and Methods: Induction of Diabetes mellitus was done by Streptozotocin (STZ) via intraperitoneal route injection. Animals were apportioned into five groups (n=5) for contrasting the activity of EC at three EC doses (25 mg/kg, 50 mg/kg, 150 mg/kg p.o.) against the standard drug (Glibenclamide) and control groups. Rats having elevated glucose levels above 250 mg/dL were considered diabetic and used for the study. Normoglycemic test, Oral Glucose Tolerance Test (OGTT), STZ-Induced diabetes, in-vitro antioxidant properties of EC extract, and in vivo antioxidant property of the serum were assessed.

Results: Phytochemistry revealed the presence of tannins, flavonoids, saponins, alkaloids, terpenoids, deoxy-sugars, and anthraquinones. The three doses of EC (150, 50, and 25 mg/kg) used in the study caused a significant decrease in blood glucose levels in the STZ-induced diabetic rat model. Also, EC (150, 50, and 25 mg/kg) produced a significant ($p < 0.001$) increase respectively in the body weight from day 0 to day 30 when compared with the untreated diabetic rat. Our results indicated that EC might be a potent free radical scavenger, as it scavenged the 2,2-diphenyl picrylhydrazyl (DPPH) radical, hydroxyl radical, and NO radicals in vitro. EC also showed a significant anti-lipid peroxidative effect in vivo. Histologic analysis revealed the regenerative impact of EC on the β -cells of diabetic rats.

Conclusion: Findings revealed that EC possess antihyperglycemic and antioxidant effects.

Keywords: *Entandrophragma cylindricum*, Diabetes mellitus, antioxidant, antidiabetic

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Introduction

Diabetes mellitus (DM) is a long-term metabolic disorder that manifests in the form of hyperglycemia as a consequence of a defect in insulin secretion and/or insulin action, along with an imbalance in the metabolism of carbohydrates, fats, and proteins. The high incidence of DM has reached epidemic proportions predominantly due to changes in lifestyle and an increase in the prevalence of obesity, cystic fibrosis, and mitochondrial defects (1). Maintenance of normal blood glucose level (BGL) is essential in diabetes since a decrease in BGL (hypoglycemia) or an increase in BGL (hyperglycemia) is a pathologic phenomenon. Hence, monitoring of antidiabetic drug therapy in the presence of other drugs is very much needed to maintain safety (2).

Entandrophragma cylindricum (*EC*) (Sprague) belongs to Meliaceae, is commonly known as West African Cedar in English and *jebo* among the natives in Southwest Nigeria. It has a significant presence in Angola, Cameroon, the Central African Republic, Congo, and Cote d'Ivoire (3). Till now, there are only two reports on the potential antidiabetic properties of *EC*, as reported by Ayoola et. al. (2017) (4), whom evaluated the methanolic stem bark extract of *EC* on alloxan-induced diabetic rats and by Balogun et. al. (2021) (5) whom assessed the aqueous extract of stem bark of *EC* on Streptozotocin-induced diabetic rats. To our knowledge, no research was found or ever reported on *EC* leaves' antioxidant and antidiabetic activities. However, we hypothesized *EC* leaves might possess a better antidiabetic and free radical scavenging property. Thus the study objectives were to investigate *EC* leaves' antioxidative and antidiabetic activities to scavenge free radicals and lower blood glucose levels. These objectives were tested in normoglycemic and STZ Induced diabetic rats using oral administration of the methanolic extract.

Materials & Methods

Streptozotocin and glibenclamide were purchased from (Sigma Chemical Company, St. Louis, MO, USA).

All chemicals and reagents required for the study were of analytical grade.

Plant Collection:

Leaves of *EC* were collected in June 2019 in Ore Forest Reserve by a local herbalist in Ore, Ondo State, Nigeria. The taxonomic identity of the bark specimen was identified and authenticated by Dr. S. A. Odewo of the Forest Research Institute of Nigeria (FRIN). Identification number (FHI: 111950) was assigned, and the sample was deposited at the herbarium. The collected leaves were shaded dried to a constant weight and pulverized with an electric grinder (Kenwood, Model BL335, Taiwan, China) and was subsequently packaged in plastic containers for further use.

Extraction of Plant Samples:

The methanolic extract was prepared by weighing 100 g of the pulverized leaves of *EC* into the Soxhlet extractor (Zhengzhou Keda machinery Co., Zhengzhou city, China), and 2 liters of 80% methanol (Matrix fine Chemicals Flums, Switzerland) was measured into a round bottom flask. The apparatus was set up, and the heater temperature was set at 65^o C, a little above the boiling point of methanol. The extraction process lasted for about 8 hours. The extract was concentrated by evaporation at 40^o C using an oven, scrapped into a universal bottle, and weighed before storage in the fridge until it was ready for use. A yield of 18.91% was obtained. The dried sample was used for further studies.

Phytochemical Screening:

Qualitative phytochemical screening of methanolic extracts of plant materials was conducted for Alkaloids (**Mayer's Reagent Test**), flavonoids, cardiac glycosides (Keller-Killiani Test), tannins, reducing sugar saponin and anthraquinones using standard methods outlined by Harborne (1973) (6), Sofowora (1993) (7), and Ayoola, et. al. (2017) (4).

Experimental animals:

26 adult male mice (22-26 g; aged 12 weeks) and Ninety-five (95) adult male rats (150- 200 g) were obtained from the Animal Centre, College of Medicine of the University of Lagos and acclimatized for two weeks. Rats were kept in polypropylene cages lined with husk in standard environmental conditions (temperature

25°C, and 12:12 h light: dark cycle). All mice and rats were fed on a standard pellet diet (Animal Care Nigeria, Ltd., Ogun State, Nigeria) and drinking water (*ad libitum*). The application for ethical clearance was made to the College of Medicine, University of Lagos Human Research Ethics Committee (CMUL/HREC), and approval to carry out the study was given with permission number (CMUL/ACUREC/01/21/925). All experiments were conducted in strict compliance with the NIH guidelines for care and use of animals.

Acute Toxicity Study:

Acute oral and intraperitoneal toxicity study of methanolic leaf extract of *EC* for determination of median lethal dose (LD_{50}) was carried out in mice. Group I served as control and received distilled water, while Group II, III, and IV and V received *EC* methanolic extracts (100 mg/kg, 500 mg/kg, 1000 mg/kg, and 2000 mg/kg, *i.p.*), respectively. For oral administration, the group I served as control and received distilled water. Group II, III, IV, and V received 100 mg/kg, 500 mg/kg, 1000 mg/kg, and 2000 mg/kg doses of *EC* methanolic extracts, respectively (8). Behavioral changes and toxic signs were observed in the mice for 24 hours and up to 14 days for delayed toxicity and mortality. Food consumption was observed daily, and body weights were documented weekly.

Experimental Design:

The antidiabetic activity of *EC* was evaluated in normal, glucose-loaded hyperglycemic and streptozotocin-induced diabetic rats. In all the studies, the animals fasted overnight for 16 h with free access to water throughout the duration of the experiment.

Evaluation of Extract on Normal Healthy Rats:

Studies in normoglycemic rats were carried out in 20 adult male rats using the procedure described by Lanjhiyana et al. (2011) (9). They were equally divided into five groups of 4 male rats in each group: 1st or normal control group received normal saline (10 ml/kg), 2nd group received 5 mg/kg of glibenclamide orally, and the 3rd to 5th groups were administered with different doses of methanolic leaf extracts of *EC* (25, 50, and 150 mg/kg, *p.o.*) respectively. Blood samples were retrieved from the tail vein before dosing (day 0) and then fasting

blood glucose tests were performed 6 hours later, on days 7, 14, and 21, respectively.

Evaluation of *EC* Extract for Oral Glucose Tolerance Test (OGTT):

OGTT of methanolic leaf extract of *EC* was carried out using the method of Mahfoud et al. (2011) (10). Thirty male adult rats were fasted overnight and were equally divided into five groups ($n = 6$). The standard control group received only 10 ml/kg normal saline, *p.o.* and the positive control group received 10 mg/kg of reference drug glibenclamide, while the three other groups were administered with different doses of methanolic leaf extracts of *EC* (25 mg/kg, 50 mg/kg, and 150 mg/kg, *p.o.*), respectively. After that, following 30 min post extract administration, the animals were fed with glucose (2 g/kg). Blood samples were retrieved from the tail vein before dosing and then at 30, 60, and 120 min after glucose administration. The fasting blood glucose levels were analysed using ACCU CHEK[®] glucometer (Roche, Mannheim, Germany) and glucose-oxidase-peroxide reactive strips (Accu-Chek, Roche Diagnostics, Germany).

Evaluation of Extract in Streptozotocin-Induced Diabetic Rats:

Diabetes was induced by a single intraperitoneal injection of 65 mg/kg of STZ, which was dissolved in cold citrate buffer, pH 4.5. The control animals received only citrate buffer. After five days of STZ injection, animals with fasting blood glucose above 250 mg/dL were termed diabetic and included in the study. The animals were randomly assigned into six groups of six animals each. They received the following treatments:

- Group I: Normal control distilled water,
- Group II: Diabetic distilled water,
- Group III: Diabetic + *EC* (25 mg/kg),
- Group IV: Diabetic + *EC* (50 mg/kg),
- Group V: Diabetic + *EC* (150 mg/kg) and
- Group VI: Diabetic + glibenclamide (10 mg/kg).

The freshly prepared *EC* extract solutions were orally administered daily for 21 days. In addition, the body weights and blood glucose level analysis were done weekly on overnight fasted animals with the aid of a portable ACCU CHEK[®] glucometer (Roche,

Mannheim, Germany) and glucose-oxidase-peroxide reactive strips (Accu-Check, Roche Diagnostics, Germany) using tail tapping method.

Sample Collection:

3 rats from each of the groups were sacrificed on the 28th day of treatment. The animals were anesthetized using cotton wool soaked in chloroform in a desiccator. The anesthetized animals were placed on a dissecting slab; the blood samples were collected from the jugular vein with lithium-heparin bottles. Blood samples were taken to the laboratory for various analyses. The most prominent lobe of the pancreas was cut off with a surgical blade and placed in a sample bottle containing 10% formalin solution for histological examination as set out by Okoye et. al. (2017) (11).

In-vitro Antioxidant Assays:

The antioxidant activity of *EC* extracts was evaluated by the phosphomolybdenum method according to the procedure reported by Prieto et. al. (1999) (12) with slight modifications.

DPPH Free Radical Scavenging Assay:

The free radical scavenging capacity of the methanolic leaf extracts of *EC* was determined using DPPH (1, 1-Diphenyl 2-Picryl Hydrazyl) as described by Patel et. al. (2012) (13) with slight modifications.

Nitric Oxide Scavenging Activities:

Nitric oxide (NO) scavenging activity was estimated by the use of the Griess-Ilosvay reaction as described by Bokhoven and Tommassen (1961) (14). For the experiment, sodium nitroprusside (10 mM) in phosphate-buffered saline was mixed with different concentrations (5 to 200 µg/ml) of methanolic extract of *EC* and dissolved in methanol and incubated at 300°C for 2 hours. The same reaction mixture without the extract but the equivalent amount of ethanol served as the control. After incubation, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H₃PO₄, and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride was immediately read at 550 nm. Inhibition of nitrite formation by the plant extracts and

the standard antioxidant ascorbic acid were calculated relative to the control. Inhibition data (inhibition percentage) were linearized against the concentrations of each extract and standard antioxidant. IC₅₀, an inhibitory concentration of each extract required to reduce 50% of the nitric oxide formation, was also determined.

Ferric Ion Reducing Capacity Assay:

Ferric ion reducing capacity assay of *EC* was carried out using the method described by Vijayalakshmi and Ruckmani (2016) with slight modifications (15).

Measurement of Extract Effect on Serum Antioxidants:

Serum Preparation:

Twenty-four hours after the last dose, the blood used for serum preparation was collected via direct heart puncture with a 21 G needle attached to a 5 mL syringe, following mild chloroform anesthesia of the rats. The serum samples were prepared using the standard method depicted by Yesufu et. al. (2010) (16). Blood samples were allowed to clot for 30 minutes and then centrifuged at 2500 rpm for 15 minutes, and sera samples were harvested.

Superoxide Dismutase (SOD) Activity:

Superoxide dismutase activity was assayed using the method of Sun et. al. (1988) (17). In this method, the xanthine-xanthine oxidase system was used to generate a superoxide flux, and Nitroblue Tetrazolium (NBT) was then used as an indicator of superoxide production. The SOD activity was expressed in units of enzyme/gram hemoglobin. One unit of SOD corresponds to the enzyme concentration required to inhibit the chromogen produced (NBT) by 50% in 1 min under standard conditions, and results were expressed as U/ml.

Estimation of Catalase Activity:

The catalase activity in serum was done using a modified method as described by Atawodi (2011) (18). 10 µL of serum was added to the test tube containing 2.80 mL of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 mL of fresh 30 mM hydrogen peroxide, and the decomposition rate of hydrogen peroxide was measured at 240 nm for 5 min

by a spectrophotometer. A molar extinction coefficient of 0.041 mM⁻¹ cm⁻¹ was used to calculate catalase activity.

Estimation of Glutathione Peroxidase (GSH) Activity:

GSH activity was measured by the method of Paglia and Valentine (1967) (19). The enzymatic reaction was initiated by adding hydrogen peroxide to the reaction mixture containing reduced glutathione, nicotinamide adenine dinucleotide phosphate, and glutathione reductase. The Shimadzu UV 1601 spectrophotometer monitored the changes in the absorbance at 340 nm. One unit of GSH is defined as micromoles of NADPH oxidized per minute. The activity was given in units per liter of plasma.

Malondialdehyde (MDA) Assay:

Lipid peroxidation levels were determined by measuring the end product of lipid peroxidation MDA with thiobarbituric acid and Shimadzu UV 1601 spectrophotometer (20). Serum MDA values were calculated using the extinction coefficient of the MDA thiobarbituric acid complex at 532 nm. MDA results were expressed as nmol/ml.

Histopathologic Assessment:

The paraformaldehyde-fixed pancreas was processed by subjecting it to the processes of clearing, dehydration, and embedding. The resulting paraffin blocks were sectioned using a microtome and stained

using Hematoxylin and Eosin stains as depicted by Pearse (1980) (21).

Statistical Analysis:

Results obtained were expressed as mean \pm SEM using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA, USA). The significance of the difference between the controls and treated groups was determined using one-way and two-way Analysis Of VAriance (ANOVA. $p < 0.05$) followed by Tukey *post hoc* multiple comparison test.

Results Phytochemical screening of EC:

Qualitative Phytochemical analysis of the methanolic leaf extract of *EC* revealed the presence of alkaloids (++) , saponins (+), triterpenes (++) , tannins (++) , flavonoids (+++), and anthraquinones (++) .

Acute toxicity:

Results from the acute oral and intraperitoneal toxicity studies showed no mortality when administered at 0 - 2000 mg/kg of methanolic extract of *EC*.

Effect of EC Treatment on Blood Glucose Level in the Normoglycemic Model:

Two-way ANOVA analysis revealed a decrease ($p < 0.05$) in the blood glucose at dose 50 mg/kg of *EC* for Day 7 by 22.9% when compared with the control baseline group. In addition, there was a decrease ($p < 0.001$) in blood glucose levels at all doses, 25 mg/kg, 50 mg/kg, and 150 mg/kg in relation to the control group on Day 21 by 14.1%, 14.7%, and 29.9%, respectively (Figure 1).

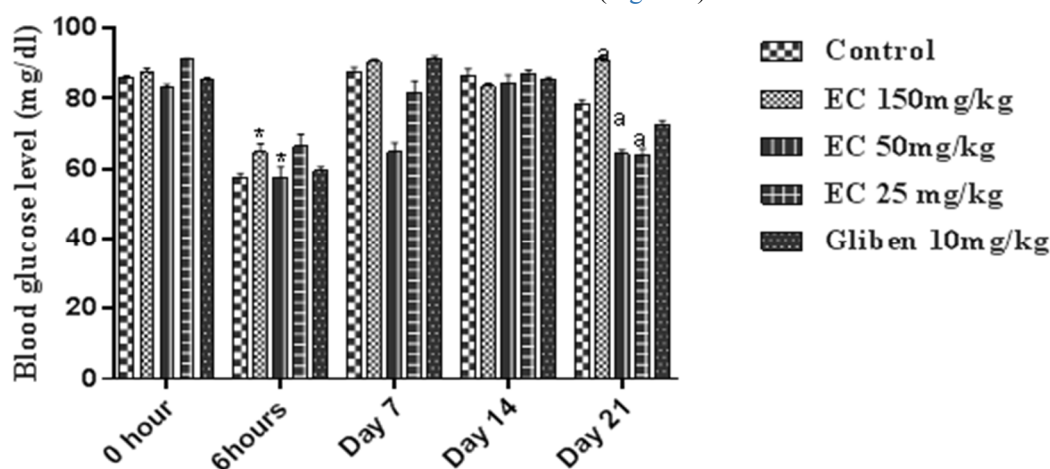


Fig 1: The effects of *E.cylindricum* treatment on blood glucose level in normoglycemic model. Values are expressed as mean \pm SEM (n=5). * $p < 0.05$, ^a $p < 0.0001$ versus control using two-way ANOVA followed by Tukey *post hoc* multiple comparison tests.

Effect of EC Treatment on OGTT:

Pre-treatments with EC (50 mg/kg) at 0 and 60 minutes ($p < 0.001$) did produce a decline in the blood glucose level following glucose administrations, while

EC (150 mg/kg) produced a 49.4% ($p < 0.05$) decrease at 120 minutes only. In the same vein, glibenclamide exhibited a decrease ($p < 0.001$) in the blood glucose levels at 0, 30, 60, and 120 minutes after glucose administration, respectively (Figure 2).

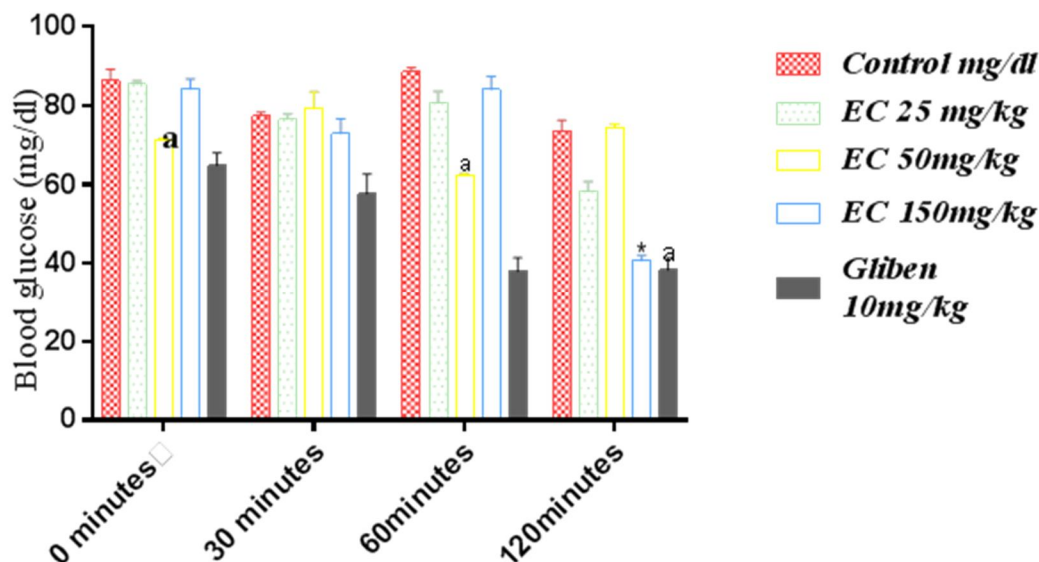


Fig 2: The effects of EC on oral glucose tolerance test. Values are expressed as mean \pm SEM (n=5). * $p < 0.05$, ^a $p < 0.001$ versus control using two-way ANOVA followed by Tukey post hoc multiple comparison test.

Effect of EC Treatment on Body Weight in the Normoglycemic Model:

After 21 days of oral administration of EC plant extract, only 50 mg/kg produced an increase ($p < 0.001$) in the body weight of animals in contrast with the control (Figure 3).

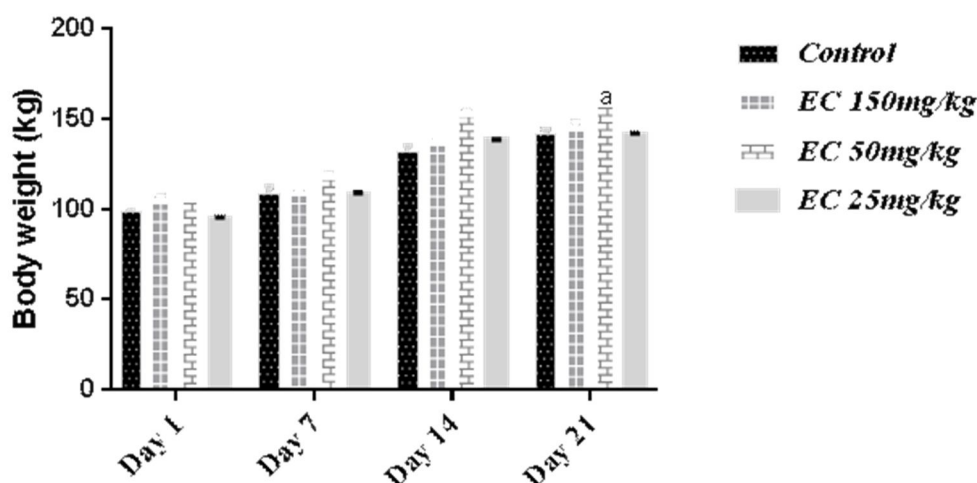


Fig 3: The effects of EC treatment on body weight in the normoglycemic model. Values are expressed as mean \pm SEM (n=5). ^a $p > 0.001$ versus control using two-way ANOVA followed by Tukey post hoc multiple comparison test.

Different levels of blood glucose of untreated diabetic animals up against various treatment groups of *EC* (150, 50, and 25 mg/kg) from day 7 to day 30 all produced a maximal decrease ($p < 0.001$) in blood

glucose by 73.2%, 60%, and 81.7%, respectively. Similarly, glibenclamide administration produced a sufficient decrease ($p < 0.01$) relative to the untreated diabetic group (Figure 4).

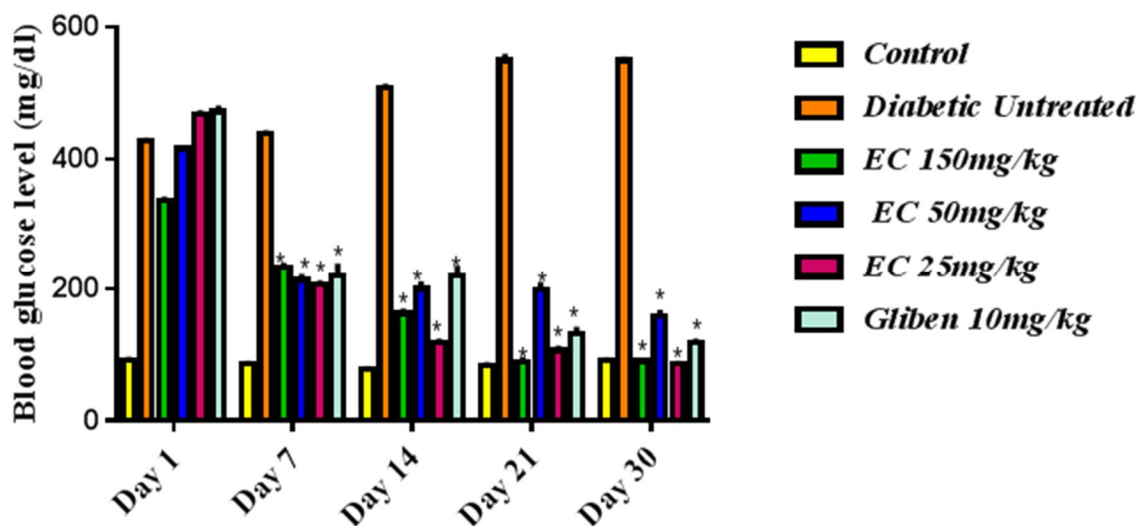


Fig 4: The effects of *EC* extract and glibenclamide on blood glucose level in 30 days treated diabetic rats. Values are expressed as mean \pm SEM ($n=5$), * $P < 0.0001$ versus diabetes untreated group using two-way ANOVA followed by Tukey *post hoc* multiple comparison test.

Effect of 30 days *EC* and Glibenclamide Treatment on Body Weight:

There was a significant ($p < 0.01$) decrease in the body weight of the untreated diabetic rats in contrast

with controls at day 21. Interestingly, *EC* (150, 50, and 25 mg/kg) all produced a significant ($p < 0.001$) increase respectively in the body weight from day 0 to day 30 when correlated with the untreated diabetic rat (Figure1).

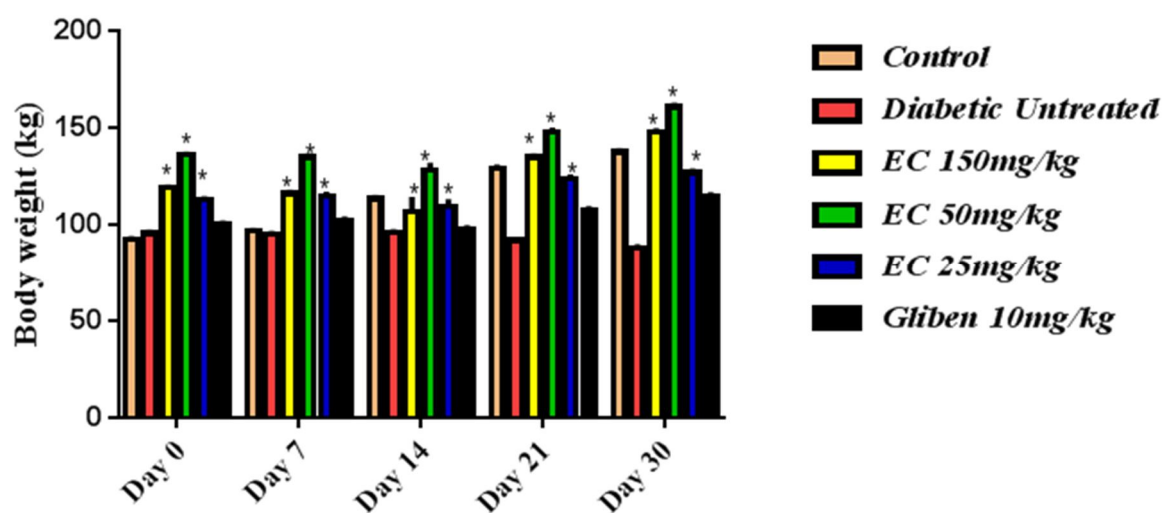


Fig 5: The effects of *EC* extract and glibenclamide on body weight in 30 days treated diabetic rats. Values are expressed as mean \pm SEM ($n = 5$), * $p < 0.001$ versus diabetes untreated group using two-way ANOVA followed by Tukey *post hoc* multiple comparisons.

In vitro Antioxidant Assay:

DPPH Free Radical Scavenging Assay:

The result showed that the values for IC_{50} were 41.82 $\mu\text{g/ml}$ for DPPH assay and 60.72 $\mu\text{g/ml}$ for *EC* and ascorbic acid, respectively. The results show that the hydrogen donating ability of *EC* was not better than the effect of the ascorbic acid reference standard (Figure 6A).

Regarding the Nitric Oxide (NO) Scavenging Activity assay, the results showed that the IC_{50} for *EC* and ascorbic acid are 43.37 and 62.80 $\mu\text{g/ml}$, respectively. These results suggest that the NO scavenging ability of *EC* was not better than the effect of the ascorbic acid reference standard (Figure 6B).

The Ferric ion Reducing Power (FRAP) assay measures the electron-donating capacity of an antioxidant. The presence of reducing agents (i.e., antioxidants) causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. The absorbance measured at 700 nm of the resultant blue-green colored solution is proportional to the amount of Fe^{2+} in the system. Therefore, an increased in absorbance is indicative of higher reducing power. The corresponding concentration of *EC* and ascorbic acid to produce a blue-green colored product of 0.5 absorbances (Effective concentration; EC_{50}) were 0.23 and 0.41 $\mu\text{g/ml}$, respectively (Figure 6C).

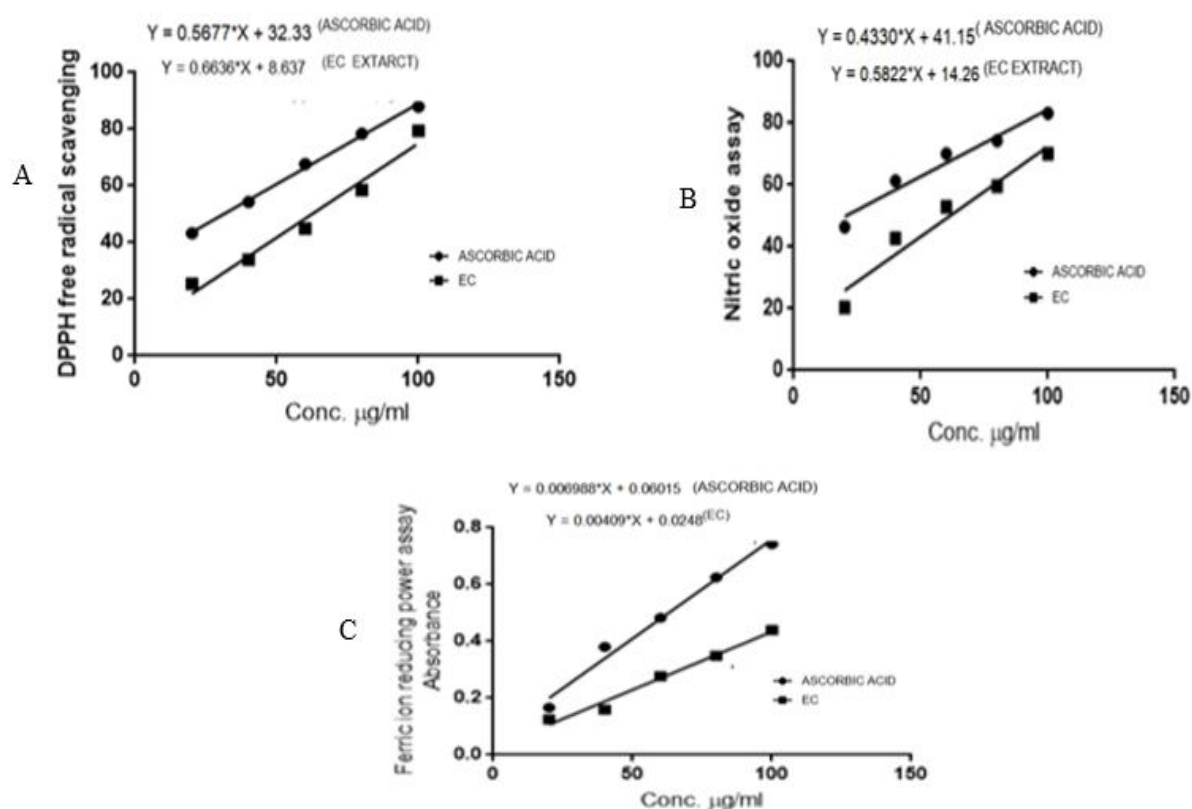


Fig 6: In Vitro Antioxidant assay comparisons of *EC* and ascorbic acid

Effect of *EC* on Oxidative Stress and Antioxidant Indices:

Lipid peroxidation levels measured as malondialdehyde (MDA) and antioxidant enzymes were assessed in normal and diabetic rats. Treatments with *EC* extracts of 150 and 50 mg/kg produced increased ($p < 0.01$) levels of GSH by 250% and 274%,

respectively, compared to the control group. Glibenclamide administration also produced a 138% ($p < 0.05$) increase in the level of GSH compared with control. Moreover, treatment with *EC* (150 mg/kg) produced a decrease ($p < 0.05$) in the level of MDA by 44.4% when compared with the untreated diabetic group (Table 1).

Table 1: Effect of *EC* on oxidative stress and antioxidant indices in normal and diabetic rats

Treatment	GSH (units/mg pro)	SOD (units/mg pro)	CAT (units/mg pro)	MDA (units/mg pro)
CONTROL	0.08 ± 0.01	0.28 ± 0.01	13.02 ± 0.53	0.08 ± 0.001
BASELINE (Untreated)	0.11 ± 0.01	0.24 ± 0.02	12.06 ± 1.56	0.09 ± 0.004
EC 150 mg/kg	0.28 ± 0.02b (-250%)	0.26 ± 0.02 (7.1%)	13.5 ± 3.25 (-3.7%)	0.05 ± 0.01 (37.5%), 44.4% ^c
EC 50 mg/kg	0.27 ± 0.00b (-274%)	0.26 ± 0.02 (7.1%)	13.6 ± 3.25 (-4.4%)	0.07 ± 0.01 (12.5%)
EC 25 mg/kg	0.1 ± 0.01 (-25%)	0.21 ± 0.003 (25%)	12.31 ± 1.15 (5.4%)	0.093 ± 0.0 (-16.25)
GLIBENCLAMIDE 10 mg/kg	0.19 ± 0.04*(-138%)	0.22 ± 0.01 (21%)	12.31 ± 1.15 (5.4%)	0.064 ± 0.00 (20%)

Values are expressed as mean ± SEM (n=5). ^bp < 0.01, ^{*}p < 0.05 versus control, ^cp < 0.05 versus untreated using two-way ANOVA followed by Tukey *post hoc* multiple comparison test. Input data in bracket represent the percentage change against the control while data without a bracket represent the percentage change against the diabetic untreated group.

Histopathological Studies:

Histology of the pancreas in experimental rats was evaluated after 30 days of *EC* treatment. Diabetic control showed degenerative and necrotic changes in the structure of Islets of Langerhans with disrupted

vasculature. Diabetic + *EC* (25 mg/kg, 50 mg/kg, and 150 mg/kg) showed the typical structure of Islets of Langerhans with disrupted vasculature while Diabetic + glibenclamide (10 mg/kg) showed the typical structure of Islets of Langerhans with disrupted vasculature (Figure 7).

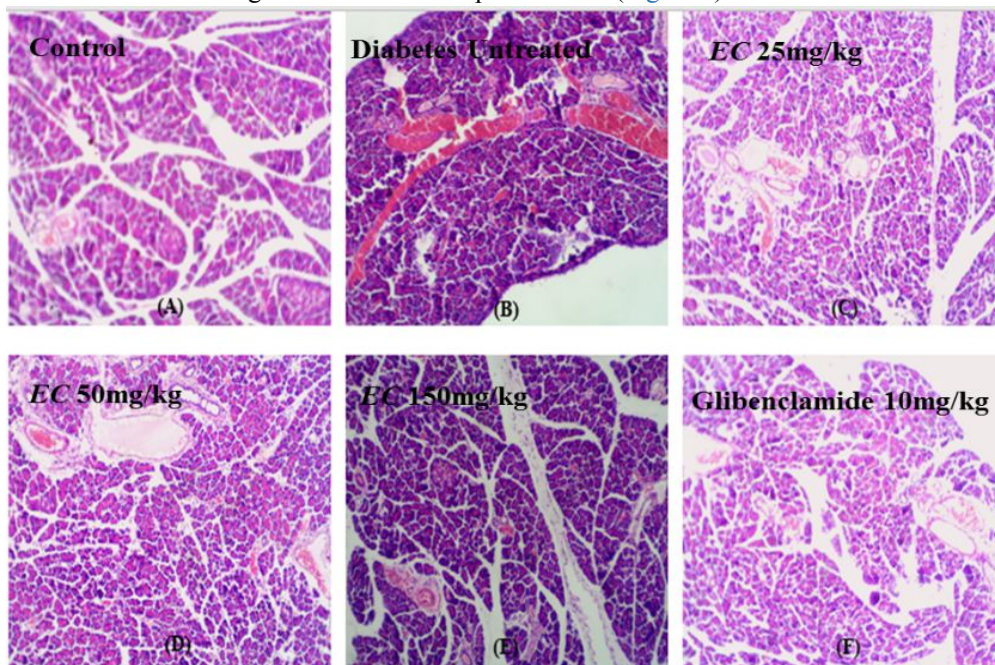


Fig 7: Sections of the pancreas: (A): Normocellular islets surrounded by normal-appearing exocrine acini with no abnormalities seen, (B): degenerative and necrotic changes in the structure of Islets of Langerhans with disrupted vasculature, (C): normal structure of Islets of Langerhans with disrupted vasculature, (D): typical structure of Islets of Langerhans with disrupted vasculature, (E): showing the typical structure of Islets of Langerhans with disrupted vasculature, and (F): showing the typical structure of Islets of Langerhans with disrupted vasculature.

Discussion

Medicinal plants continue to provide valuable therapeutic agents in both modern medicine and traditional systems (22), and natural product derived drugs have been proven to play a vital role in the management of diabetic diseases (23). The phytochemical screening of *EC* showed the presence of saponins, terpenoids, tannins, deoxy-sugars, anthraquinones, and alkaloids. The results of this study were inconsistent with the results of Balogun et. al. (2021) (5), who worked on the stem bark of this plant but revealed an absence of saponin and alkaloids. Saponins have been reported as plant phytochemical having insulin sensitization and anti-hyperlipidemic effects in the diabetic state (24). Flavonoids and alkaloids have also been reported to constitute active biological principles of most medicinal plants with hypoglycemic and antidiabetic properties (25). Further studies are required to isolate, identify, and characterization of the active phyto-constituents and determine the precise mechanisms of actions responsible for the excellent antidiabetic effects elicited by *EC*. Any of these mentioned compounds could have induced the observed effects. Many plants that have high contents of saponins and terpenoids have been reported to possess high antidiabetic activities (24).

Acute oral toxicity testing is the initial test carried out to evaluate the toxicity profile of any plant or substance (26). This is to unearth deleterious reaction to either a single dose or an overdose of a particular plant or substance (27). In our study, *EC* did not produce any mortality at 2000 mg/kg for both oral and intraperitoneal administration, and this dosage did not in the very least alter the behavioral patterns of the animals. This vehemently suggests that the intraperitoneal and oral LD50 of *EC* leaf extract is far greater than 2000 mg/kg body weight and can be considered to be non-toxic (8). The excellent safety value discovered in this study may suggest the reason why *EC* is a widely used medicinal plant. As observed in our study, *EC* (50 mg/kg) exhibited a significant decrease in the blood glucose level at day 7 and day 21, which was within the normal glycemic state after subacute exposure of

normoglycemic rats to plant extract. This implies that *Entandrophragma cylindricum* plant extract does not tend to cause hypoglycemia under normal physiological conditions, unlike some antidiabetic drugs which cause drug-induced hypoglycemia (28).

Achieving sustainable blood glucose control is vital to ward off the numerous complications of diabetes and improve patients' wellbeing. Hence a consistent reduction in hyperglycemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macro-vascular complications. Thus the glucose-induced hyperglycemic model was selected to evaluate for the antihyperglycemic activity of *EC*. Any substance that is effective in diabetes will indeed have the ability to control the rise in glucose level by various mechanisms, and the ability of the extracts to prevent hyperglycemia could be determined by a glucose-loaded hyperglycemic model (29). The *EC* (50 mg/kg) at 0 and 60 minutes produced a significant decrease in the blood glucose level following glucose administration. While *EC* (150 mg/kg) produced a 49.4% decrease at 120 minutes, glibenclamide exhibited a decrease in the blood glucose levels at 0, 30, 60, and 120 minutes after glucose administration, respectively.

It is a known that after glucose administration in OGTT, the elevated glucose concentration in the blood induces the secretion of insulin. The secreted insulin will then stimulate peripheral glucose consumption and control the production of glucose through various mechanisms. Insulin reduces blood glucose in about 2 to 3 h to bring back the glucose level to normal (30). Our findings with our extract and glibenclamide support this. The antihyperglycemic activity of the methanolic leaf extract of *EC* could be due to a beneficial effect of the active constituents on carbohydrate metabolism in glucose-loaded rats. The effect of glibenclamide, the reference standard used in this study, on glucose tolerance has previously been as a result of its enhanced activity of β -cells in the pancreas resulting in more insulin secretion. So, the mechanism behind this antihyperglycemic activity of our extract, at differing doses, involves an insulin-like effect, probably through peripheral glucose consumption or increasing the

sensitivity of β -cells to glucose, which will result in insulin release. *EC* has been reported to possess antidiabetic effects and antioxidant properties (4). This present study showed that *EC* leaf extract could be a beneficial antidiabetic agent through attenuation of hyperglycemia via stimulating pancreas healing, scavenging free radicals, and mitigating the reduction of body weight which is one of the conditions seen in diabetic Mellitus. Renewal of the beta cells of the pancreas in this study has been well studied in various animal models. The injection of streptozotocin has been found to destroy the cells and also create an imbalance between the renewal and loss of these cells. It was found that streptozotocin-untreated diabetic rats had a higher level of blood glucose compared with our control group. However, our results revealed that treatment with *EC* leaf extract for 30 days mitigates the hyperglycemic and destructive effects of streptozotocin in the pancreas, possibly by acting on the surviving beta cells of the pancreas to release insulin or by stimulating the regeneration of the pancreatic beta cells. Hence it can be deduced that *EC* is compelling and shows similar curative effects as glibenclamide. Our findings clearly show that *EC* (25, 50, and 150 mg/kg) significantly reduced the blood glucose level when compared to the streptozotocin untreated diabetic rats. Interestingly, *EC* 25 mg/kg produced more effective antidiabetic effects than other doses administered. Our finding is consistent with Balogun *et. al.* (2021) (5), who evaluated the antidiabetic effect of the plant's stem bark. Decreased body weight has been a well-implicated condition found in diabetics. In our present study, there was a significant decrease in the body weight of streptozotocin untreated diabetic rats. Interestingly, a change in body weight was observed to be significantly increased from day 0 to day 30 in all the treatment groups (25, 50, and 150 mg/kg). This unique finding may serve as another therapeutic advantage of *EC* in combating weight loss in diabetic conditions.

Oxidative stress plays a role in the development of diabetes complications, both microvascular and cardiovascular. The metabolic abnormalities of diabetes cause mitochondrial superoxide overproduction in

endothelial cells of large and small vessels and the myocardium (31). Our results indicated that different constituents of *EC* might be a potent free radical scavenger, as they scavenged the DPPH radical, hydroxyl radical, and NO radical in in-vivo chemical systems. *EC* plant extract reacted with DPPH radical and converted it to 1, 1-diphenyl-2-picrylhydrazine, and the degree of discoloration (indicated by a decrease in absorption) indicated the scavenging activity. The strong scavenging capacity of the extracts of *EC* on DPPH' might be due to the hydrogen donating ability of the polyphenolic compounds present (evident in its phytochemical screening) in the extracts.

The ferrous ion increases lipid oxidation through the breakdown of hydrogen and lipid peroxides to reactive free radicals via the Fenton reaction. It can also accelerate peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals. These radicals can themselves attract hydrogen and perpetuate the chain reaction of lipid peroxidation (32). In this method, ferrozine can quantitatively form complexes with Fe^{2+} and in the presence of the extracts, the complex formation is inhibited. Therefore, minimizing ferrous ions by the extracts may afford protection against oxidative damage. Our study found a correlation between the polyphenolic content and the antioxidant activity, as the methanolic leaf extracts of *EC* contained high phenolic and flavonoid content and exhibited higher antioxidant activity. Various studies have explained the implication of oxidative stress in diabetes mellitus and the complications associated with it (33). GSH has been reported to be the significant endogenous antioxidant and directly involved in the neutralization of Reactive Oxygen Species (ROS) (34). It is hugely involved in the maintenance of exogenous antioxidants in their reduced (active) forms (34). Lipid peroxidation (LPO) is a process in which oxidants such as free radicals cause cell damage. Thus, an increase in LPO will increase destruction caused by oxidation and has been well implicated in diabetes mellitus (35). GSH and LPO are known as well-used markers for measuring oxidative stress (36). Treatment with Glibenclamide and *EC* (50 and 150 mg/kg) significantly increased the level

of GSH when compared with the control group. However, treatment with *EC* (150 g/kg) decreased the level of lipid peroxidation when compared with the streptozotocin untreated diabetic group. Therefore, the observed increased activity of the GSH and diminished amounts of MDA, which is a lipid peroxidation marker, in the *EC* treated animals indicates that the extract had antioxidant protective potentials.

Conclusion

The results obtained from this study demonstrate that the methanolic leaf extract of *EC* has both antidiabetic and antioxidant activities and could be a potential agent in the management of diabetes. This present investigation has also opened an avenue for further research, especially regarding the development of potent formulations for diabetic mellitus from *EC* extract. Further extensive investigations are necessary to determine the exact phyto-constituents responsible for its possible antidiabetic effects.

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Authors' Contributions

FO, EI, and OK planned the experimental procedure and collected the data needed for the study. FO, EI, OK, and OT performed the data analysis. FO and OK composed the article. All the contributors thoroughly read and approved the final manuscript.

Conflict of interests

There are no conflicts of interest.

References

1. Dhodi JB, Mestry SN, Juvekar AR. Diabetic nephropathy-genesis, prevention, and treatment. *Int J Pharm Pharm Sci* 2014;6(9):42-7.
2. Kumar KE, Rani PJ, Ram KR, Swathi P, Gupta M. Pharmacodynamic and pharmacokinetic drug interaction of gliclazide and risperidone in animal models. *Int J Pharm Pharm* 2012;4:659-62.
3. Mbula J, Kwembe J, Tshilanda D, Ngobua K, Kabena O, Nsimba S, et al. Antisickling, antihemolytic and radical scavenging activities of essential oil from *Entandrophragma Cylindricum* (Sprague) Sprague (Meliaceae). *J Adv Med Life Sci* 2018;6(2):1-5.
4. Ayoola M, Akinwunmi K, Agboola O. Anti-diabetic and Antioxidant Activities of *Entandrophragma cylindricum* and *Triclisia subcordata*. *Niger J Nat Prod Med* 2017;21:24-31.
5. Balogun EA, Ayeni SD, Otolorin A-AO, Rotimi DE. Anti-diabetic and Antidyslipidemic Activities of *Entandrophragma Cylindricum* Extract on Streptozotocin-Induced Diabetic Rats. *Biointerface Res Appl Chem* 2021;11(6):14251-9.
6. Harborne A. *Phytochemical methods a guide to modern techniques of plant analysis: springer science & business media*; 1998.
7. Sofowora A. *Phytochemical Screening of Medicinal plants and traditional medicine in Africa: Spectrum Books Limited, Nigeria*; 1993. pp: 150–156.
8. Lorke D. A new approach to practical acute toxicity testing. *Arch Toxikol* 1983;54(4):275-87.
9. Lanjhiyana S, Garabadu D, Ahirwar D, Bigoniya P, Rana A, Patra K, et al. Antidiabetic activity of methanolic extract of stem bark of *Elaeodendron glaucum* Pers. in alloxanized rat model. *Adv Appl Sci Res* 2011;2(1):47-62.
10. Mahfoud F, Schlaich M, Kindermann I, Ukena C, Cremers B, Brandt MC, et al. Effect of renal sympathetic denervation on glucose metabolism in patients with resistant hypertension: a pilot study. *Circulation* 2011;123(18):1940-6.
11. Okoye N, Monanu MO, Ohanehi VO. Combined Effects of Aqueous Extracts of *Tetracarpidium Conophorum* (Walnuts) and *Vernonia Amygdalina* (Bitter leaves) on the Pancreas and Kidney of Alloxan Induced Diabetic Wistar Rats. *JASEM* 2017;21(5):893-901.
12. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation

- of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem* 1999;269(2):337-41.
13. Patel R, Patel A, Desai S, Nagee A. Study of secondary metabolites and antioxidant properties of leaves, stem and root among *Hibiscus rosa-sinensis* cultivars. *Asian J Exp Biol Sci* 2012;3(4):719-25.
 14. Bokhoven C, Tommassen P. Continuous determination of traces of nitric oxide. *Nature* 1961;190(4774):435-6.
 15. Vijayalakshmi M, Ruckmani K. Ferric reducing antioxidant power assay in plant extract. *Bangladesh J. Pharmacol* 2016;11(3):570-2.
 16. Yesufu H, Bassi P, Khan I, Abdulrahman F, Mohammed G. Phytochemical screening and hepatoprotective properties of the aqueous root bark extract of *Sarcocephalus latifolius* (Smith) Bruce (African Peach). *Arch. Clin. Microbiol* 2010;1(2).
 17. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin. Chem* 1988;34(3):497-500.
 18. Atawodi SE. Evaluation of the Hypoglycemic, Hypolipidemic and Antioxidant Effects of Methanolic Extract of " Ata-Ofa" Polyherbal Tea (APolyherbal) in Alloxan-Induced Diabetic Rats. *Drug Invent. Today* 2011;3(11).
 19. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967;70(1):158-69.
 20. Hunter M, Nlemadim B, Davidson D. Lipid peroxidation products and antioxidant proteins in plasma and cerebrospinal fluid from multiple sclerosis patients. *Neurochem. Res* 1985;10(12):1645-52.
 21. Atkinson D. *Histochemistry: Theoretical and applied* (Everson Pearse, AG): ACS Publications; 1954.
 22. El-Demerdash F, Yousef MI, Abou El-Naga N. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. *Food Chem. Toxicol* 2005;43(1):57-63.
 23. Gulati V, Harding IH, Palombo EA. Enzyme inhibitory and antioxidant activities of traditional medicinal plants: potential application in the management of hyperglycemia. *BMC Complement Altern. Med* 2012;12(1):1-9.
 24. El Barky A, Ali E, Mohamed T. Marine sea cucumber saponins and diabetes. *Austin Pancreat Disord* 2017;1(1):1-7.
 25. Odetola A, Akinloye O, Egunjobi C, Adekunle W, Ayoola A. Possible Antidiabetic And Antihyperlipidaemic Effect Of Fermented *Parkia Biglobosa* (Jacq) Extract In Alloxan-Induced Diabetic Rats. *Clin. Exp. Pharmacol. Physiol* 2006;33(9):808-12.
 26. Akhila JS, Shyamjith D, Alwar M. Acute toxicity studies and determination of median lethal dose. *Curr. Sci* 2007;917-20.
 27. Klein R. *Toxicology and herbs: an introduction*. *Aust. J. Medical Herbal* 1996;8(4).
 28. Dada OK, Akindele AJ, Morakinyo OA, Sofidiya MO, Ota D. Hypoglycemic and antioxidant activities of the hydroethanolic leaf extract of *Byrsocarpus coccineus* Schumach. & Thonn.(Connaraceae). *Chin. J. Nat. Med* 2013;11(6):628-37.
 29. Jarald E, Joshi SB, Jain DC. Biochemical study on the hypoglycaemic effects of extract and fraction of *Acacia catechu* willd in alloxan-induced diabetic rats. *Int J Diabetes Metab* 2009;17:63-9.
 30. Jarald E, Joshi S, Jain D. Antidiabetic activity of aqueous extract and non polysaccharide fraction of *Cynodon dactylon* Pers. *Indian J Exp Biol* 2008 Sep;46(9):660-7.
 31. Korshunov SS, Skulachev VP, Starkov AA. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Lett* 1997;416(1):15-8.
 32. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of *Spondias pinnata*. *BMC Complement Altern. Med* 2008;8(1):1-10.
 33. Ugochukwu NH, Cobourne MK. Modification of renal oxidative stress and lipid peroxidation in streptozotocin-induced diabetic rats treated with extracts from *Gongronema latifolium* leaves. *Clin. Chim. Acta* 2003;336(1-2):73-81.
 34. Scholz R, Graham K, Gumprich E, Reddy C. Mechanism of interaction of vitamin E and glutathione in the protection against membrane lipid peroxidation. *Ann. N. Y. Acad. Sci* 1989;570(1):514-7.
 35. Davi G, Chiarelli F, Santilli F, Pomilio M, Vigneri S, Falco A, et al. Enhanced lipid peroxidation and platelet

activation in the early phase of type 1 diabetes mellitus: role of interleukin-6 and disease duration. *Circulation* 2003;107(25):3199-203.

36. Onyema OO, Farombi EO, Emerole GO, Ukoha AI, Onyeze GO. Effect of vitamin E on monosodium glutamate induced hepatotoxicity and oxidative stress in rats. *Indian J Biochem Biophys* 2006 Feb;43(1):20-4.