Original Research Article

ANTI-DIABETIC EVALUATION OF METHANOLEXTRACT OF FICUS EXASPERATA VAHL. (MORACEAE)

ABSTRACT

Background
Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from impairments in insulin secretion, insulin action or both. The side effect of orthodox is alarming and expensive.This prompted a search for a cheaper and reliable alternative drug medicinal plant for the management of diabetes.
Aim
This study aimed at carrying out anti-diabetics evaluation of methanol extract of *Ficusexasperata*leaves.
Methology
The cold maceration method was used to obtain the extract using methanol as solvent. Lorkes method was used to carry out the acute toxicity studyon mice, alloxan induced model was used to evaluate the antidiabetic potentialson diabetic rats and blood glucose level was measured using glucometer and statistically analyzed.
Result
Acute toxicity study indicated no mortality or any behavioral changes even at 5000mg/kg body weight. The extract at 200mg/kg and 400mg/kg exhibited anti-diabetics activity in dose dependent manner. 400mg/kg body weight reduces blood glucose level significantly at p value less than 0.05 when compared to the standard drug.
Conclusion
The methanol extract of *Ficusexasperata* leaves have demonstrated a reasonable antidiabetics activity. The research has given an insight in the use of the plant leaves in controlling blood sugar level and substantiate its folkloric uses. Further studies on isolation of bioactive compound,establishment of the mechanismof actionfor the observed anti-diabetic activity and hepato-renal and hematological effects are recommended.

Keywords: Diabetes, Phytochemicals, Medicinal Plants, and blood glucose level

Introduction

Diabetes mellitus is a disease caused by chronic hyperglycaemia due to defects in insulin secretion, insulin action or both [1]. Diabetes disorder is characterized by frequent urine, presence of glucose in urine and hyperglycemia [2]. There are two major clinical manifestation of diabetes, the type 1 diabetes or insulin dependent diabetes mellitus (IDDM) and type 2 diabetes or non-insulin dependent diabetes mellitus (NIDDM). Based on pathophysiology, type 2 diabetes mellitus is the predominant form of the disease mostly as a

result of consumption of diets that promote obesity coupled with physical inactivity. Globally, type 2 diabetes mellitus is considered one of the most rapidly growing non-communicable diseases with high mortality rate [3].

According to the International Diabetes Federation (IDF) 2021 updates, approximately 536 million adults between ages 20-79 are living with diabetes while undiagnosed diabetes cases are estimated to be 183 million. The prevalence is projected to rise to 642 million adults by 2030 and 783 million by 2045. In Nigeria, there are estimated3.6 million diabetic cases, with another 1.9 million undiagnosed cases between ages 20-79 years. It is projected to increase to 4.9 million by 2030 and 7.9 million by 2045 [4].

Currently, diabetes therapy is based on the use of hypoglycemic drugs (sulfonamides, biguanides, and insulin), on Diet measures, exercise, and requires a lifelong treatment. Considering the level of poverty in underdeveloped and developing countries, the need for a better and cheaper medication cannot be over emphasized. Medicinal plants have always provided a cheaper and time trusted alternative for the treatment and management of various diseases over time.

Medicinal plants have continued to attract attention in global search for effective methods of using plants parts: seeds, leaves, stem and root barks for many diseases affecting humans [5]. They are playing a major role in the treatment of various diseases and ailment in humans and animalssince days immemorial with proven efficacy [6]. However, their acceptability by some cultures of the world has been limited by lack of specific dose regimen and thorough scientific information on toxicity profile to validate their safety [7].

F. exasperate Vahl. Belong to the family of moraceae, a terrestrial tropical shrub or small tree with scabrous, with ovate leaves that grows up to about 20m tall and prefers evergreen and secondary forest habitats. It is widespread in tropical Africa [8]. Traditionally different parts of this plant (fruit, leaf, sap, bark, and root) are considered medicinally important. They are

used as analgesic, diuretic, vermifuges, febrifuge, abortifacient, wound healing, leprosy, ophthalmic and oral infections, nasopharyngeal afflictions, arthritis, rheumatism, gout, edema, kidney disorders, diarrhea, dysentery, hemorrhoids, venereal diseases and animal fodder [9]. Previous researchers have reported that *F. exasperata* leaves possesses anti-inflammatory, antioxidant, antiulcer, anticonvulsant, antiarthritic, antimicrobial and hypotensive properties [11-14].

However, this study was carried out to evaluate methanol extract of *F. exasperata*for in vivo anti-diabetic potentials on alloxan induced diabetic rats

Material and methods

Plant Sample collection

The leaves of *F. exasperata*was collected from Agbani in Nkanu West Local Government Area of Enugu State, Nigeria on November, 2021. It was identified by a Taxonomist, MrOzioko Alfredof the International Centre for Ethnomedicine and Drug Development (InterCEDD), 110 Aku road Nsukka Enugu state and herbarium sample was deposited in theresearch Centre.

Animals

Twenty-eight (28) apparently healthy male and female rats of body weights ranging between 57-144g and 12 Wister albino mice, weighing 17-27g were used for the experiment. The animals were sourced from animal house of the Department of Pharmacology, Enugu State University of Science and Technology, Agbani Enugu under standard conditions. They were housed in aluminium cages in soft wood shavings as beddings in a room, at room temperature with free access to drinking water and ratchow food ad libitium. Therats were acclimatized to normal laboratory conditions prior to study.

Preparation of plant sample

The fresh plant sample was collected from the bush, and the good leaves were separated from the unwanted parts of the plants. The fresh leaves samples were then washed under a running tap water to remove dust particles and other contaminants. The leaves were air dried under a shade at room temperature for 14 days. Thereafter the leaves were pulverized to a fine course powder using an electrical blender.

Extraction

A 400g of the air dried, finely pulverized leaves sample was soaked in 2.5litres of methanol and left for 72 hours at room temperature of $25^{o} \pm 2^{o}$Cwith constant agitation. The extract was filtered first, using a fine grade cloth and second filtration was done using funnel clogged with cotton wool. The filtrate was concentrated using rotary evaporator under reduced temperature to obtain methanol extract. The percentage yield of the extract was determined and then transferred into an air-tight container and stored at $4^{o} \pm 2^{o}$C in a refrigerator until when needed.

Acute Toxicity Study

This was performed according to method described previously [18]. 12 mice were used after acclimatization. An initial investigation involving administering (10, 100 and 1000 mg/kg) of the plant extract to three different groups of three mice each. After 24 hours and there was no death recorded. In this second stage, three dose levels were used (1600, 2900 & 5000 mg/kg) and physical observations were made up to 24 hours for mortality and behavioral changes

The alloxan-induced diabetes model

The baseline blood glucose levels of the rats and weight were determined before they were induced with diabetes by intra peritoneal (IP) injection of 150mg/kg body weight of alloxan monohydrate solution. After 48 hours, the glucose levels of the rats were checked, the rats had increased glucose level using a glucometer. The diabetic rats were regrouped into 5

animals per cage in 4 different cages. The treatments were given to the rats in each group with a known anti-diabetic drug (positive control), the plant extract (200mg and 400mg) and normal saline (negative control). The blood glucose level of the diabetic rats was checked at 0 days (baseline), 4 days, 8 days and 12 days during the treatment period. The treatment lasted for 12 days after which the rats were sacrificed.The animals were grouped into 4 as follow:Group I- Negative Control rats received normal saline, Group II - Positive control treated with 5mg/kg body weight glibenclamide, Group III- Treatment with 200mg/ kg. body weight methanol extract, and Group IV- Treatment with 400mg/ kg. body weight methanol extract

Statistical analysis

The data obtained was analyzed using SPSS version 23 and the significance difference between the control and treated groups determined using one-way analysis of variance (ANOVA) followed by turkey t-test. P values less than 0.05 were considered to be statistically significant.

RESULTS

Percentage Yield of the Extract

The extract gave a percentage yield of 4.5%.

Weight of crude sample = 400g

Total yield of the extract = 18.26g

Percentage yield = total yield / weight of the crude sample × 100

= 18.26g / 400g × 100

= 4.5%

Acute Toxicity Study

The acute toxicity study result obtained showed no mortality in both phase 1&2 experiment as presented in Table 1and 2.

Table 1: phase 1 acute toxicity test

Samples	Dose 10mg/kg	Dose 100mg/kg	Dose 1000mg/kg
Methanol extract	0/3	0/3	0/3

Table 2: phase 2 acute toxicity test

Dose level	1600 mg/kg	2900 mg/kg	5000 mg/kg
Surviving rat	3/3	3/3	3/3

Effect of Extract on the Blood Glucose Level of alloxan Induced Diabetic Rats

The extract at 200mg/kg and 400mg/kg exhibited antidiabetics activity in dose dependent manner. 400mg/kg body weight reduces blood glucose lowering effect significantly at $p < 0.05$ when compare to standard drug at 5mg/kg body weight as presented in table 3.

Table 3. Effects of *F. exasperata* leaves extract on blood glucose level of alloxan induced diabetic rats

Groups	Mean Anti-diabetic response per Day					
	IGLBE	Weight	0 day (GLAI)	Day 4	Day 8	Day 12

Negative control N. Saline	108.6±0.5	111.8±0.24	345.6± 0.37	215.2± 0.19	172.8± 0.12	114.8± 0.48
Positive control Glibenclamide 5mg/kg	112.8± 0.6	81.2± 0.67	500.4± 0.27	327.4± 0.3	211± 0.7	146± 0.23
200mg/kg extract	114.2±0.12	95.8± 0.84	369.2± 0.4	237.6± 0.23	137.6± 0.73	106.6± 0.42
400mg/kg extract	115± 0.11	122 ± 0.63	438.8± 0.47	267± 0.18	149.2± 0.68	89.4± 0.28*

Data are presented as Mean ± SEM; superscripts * represents the level of significance at $p<0.05$ when compared to positive control. Repeated Measures ANOVA followed by turkey, test n=5

IGLBE = Initial Glucose Level before Induction, GLAI = Glucose Level after Induction, Positive control (Glibenclamide), Negative control N. saline = Normal saline

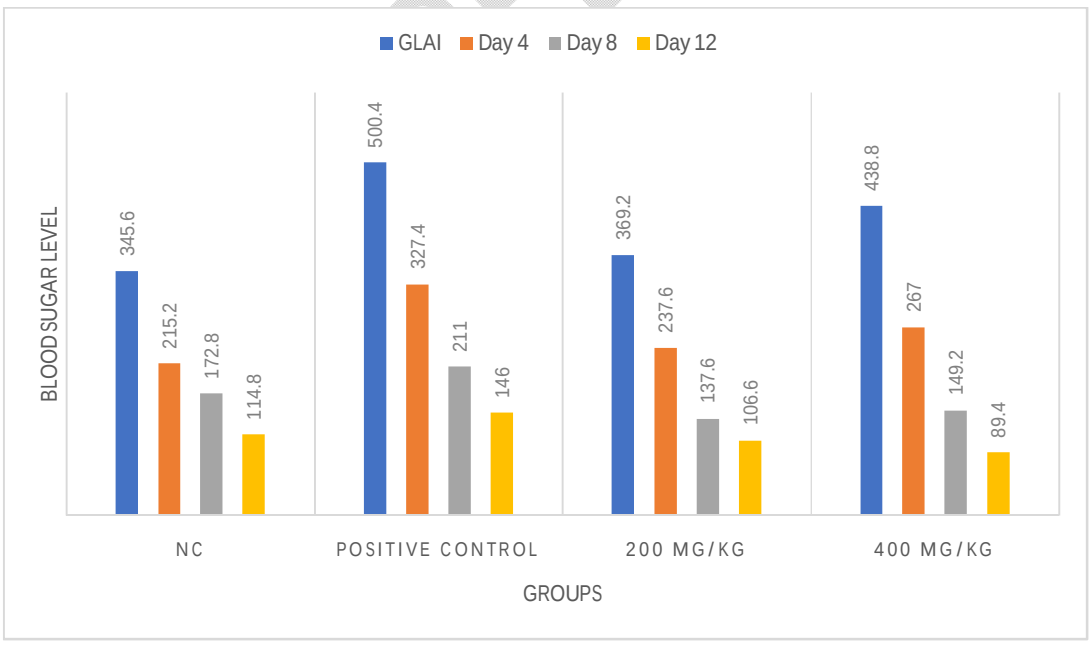

Fig: 1 Effect of extract and standard drug on experimental animals

Discussion

This present research has shown that the methanol extract of *F. exasperata*leave possess antidiabetics potentials due to its effect in lowering the blood glucose level of the alloxan induced diabetic rats. The effect is attributed to the phytochemical constituent such as phenols,flavonoids, alkaloids, tannins, saponins, steroids and triterpenespresent in the plant extract as previously report [11- 18]. The result of acute toxicity study of extract orally administered showed no mortality at doses less or equal to 5000mg/kg(table 1 and 2) which is in line with previous study [19]. This suggests that the methanol extract is safe for ethnopharmacological research buthepato-renal and hematological parameter must be established before administering it to humans.

The methanol leaves extract of *Ficusexasperata*significantly decreased the blood glucose level as observed in this present study(table 3 and fig. 1). At 400mg/kg body weight there is a significant difference at $p< 0.05$ when compared to standard drug whereas no significant difference occurred between 200mg/kg dose and standard drug. The result corroborates earlier findings that established the blood sugar lowering effect of aqueous leaves extract of *Ficusexasperata*[20-22]. This observed activity could be present of flavonoids, steroids,saponins, terpenoids and tannins in different plant parts which have been reported [23]. From our findings, as the treatment progresses a significant decrease is observed due to increase in the concentration of extract administered. Therefore, the antidiabetic activity of the methanol extract follows dose dependent manner. It is worthy of note that the use of the plant should be monitored at interval as there is tendency of causing hypoglycemic condition when consumed in large amount. We recommend 200mg of the methanol extract as optimum dose to be administered. The significant decrease in blood glucose levels suggested that the extract contains therapeuticphytoconstituents withantidiabetic potentials. It has been established that the possible mechanism by which plant extract bring about its blood glucose lowering action is by potentiating the insulin effect and by increasing the pancreatic secretion

of insulin from β cell. This lowering blood sugar effect observed might be the synergic action of the phytochemical constituents present in *F. exasperate* leaves. The results obtained from this study suggest that the methanol leaves extract of *Ficus exasperate* contained bioactive constituents that may have relevant antidiabetic properties used in the management of diabetes mellitus.

Conclusion

The methanol extract of *F. exasperata* leaves has significant anti-diabetic activity. This is as a result of present of phytochemical constituent with proven therapeutic potentials. The study also established safety of the leaves extract with respect to doses administered. These observations justified the folkloric use of the plant in management of diabetic diseases. Further studies are needed to establish the hepato-renal and hematological effects of the methanol extract and also to isolate the active ingredient responsible for the observed anti-diabetic activity of the leaves extract.

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