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Research Article

ANTIDIABETIC AND ANTIHYPERLIPIDAEMIC EFFECTS OF THE ETHANOL EXTRACT OF THE LEAVES AND STEM OF *CISSUS GRACILLIS* (GULL ET PERR) (*VITACEAE*)

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ABSTRACT

Objectives: This study investigated the antidiabetic and antihyperlipidemic potential of the ethanol extract of the leaves and stem of *Cissus gracillis* on alloxan monohydrate-induced diabetic albino rats.

Methods: Preliminary phytochemical screening and acute toxicity were carried out. Animals were assigned into seven groups of five rats each. Groups A and B were administered 10 mg/kg each of glibenclamide and atorvastatin respectively, C, D, and E were given 125, 250 and 500 mg/kg of ethanol extract of *C. gracillis,* respectively, daily for 21 days through oral gavage, group F was diabetic but untreated (diabetic control group), while group G was non-diabetic and untreated which served as the control group.

Results: Phytochemical screening revealed the presence of steroids/triterpenoids and carbohydrates. LD_{50} was above 5000 mg/kg. The extract at 500 mg/kg showed a statistically significant ($p<0.05$) decrease in blood glucose level when compared with the glibenclamide group on day 21. However, gradual non- significant reduction in blood glucose levels were observed in the extract treated groups on the 7^{th}, 14^{th}, and 21^{st} days of treatment. The administration of ethanol extract of *C. gracillis* to alloxan-induced diabetic rats produced a decrease in total cholesterol, triglycerides, and low-density lipoproteins comparable to glibenclamide and atorvastatin.

Conclusion: The ethanol extract of the leaves and stem of C. *gracillis* possess a mildly significant antidiabetic and antihyperlipidemic activity.

Keywords: Antidiabetic, Anti-hyperlipidaemic, Ethanol, *Cissus gracillis*, Alloxan, Glibenclamide, Atorvastatin.

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INTRODUCTION

Diabetes mellitus is defined as a metabolic disorder characterized by a persistent elevation of blood glucose levels associated with absence or inadequate insulin secretion, with or without concurrent impairment of insulin action. Diabetes is usually classified according to the etiology, by far the most common two being type 1 and type 2 diabetes mellitus [1]. Diabetes is a global health problem and accounted for 1.5 million deaths in 2019 [2]. Several pathogenic processes are involved in the development of diabetes, ranging from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia [3].

Besides hyperglycemia, other symptoms such as hyperlipidemia are involved in the development of microvascular complications of diabetes, which are the major causes of morbidity and death [4]. The World Health Organization has also substantiated the utilization of herbal remedies for the management of diabetes [5].

Management of diabetes without hyperlipidemia and various other associated side effects is still a challenge to the medicinal community. Combination of allopathic and herbal drugs may help to reduce the resistance to insulin and/or oral hypoglycemic therapy and the associated side effects. Hence, the continued research on medicinal plants which may serve as templates or leads for newer drugs.

Abnormalities in lipid profile are one of the most common complications in diabetes mellitus, which is found in about 40% of diabetes. The level of serum lipids (cholesterol, triglycerides, low-density lipoprotein) is usually elevated in diabetes mellitus and such an elevation represents the risk factor for the development of coronary heart disease [6]. *Cissus gracillis* is an herbaceous, perennial climbing plant producing slender stem up to 7.5 m long. The stem crumbles over the ground, climbing into the surrounding vegetation and attaching themselves by the use of tendrils. The plant is sometimes harvested from the wild, mainly as a medicinal, but sometimes also as food [7]. It is known as Anaya or Yaakuwarfataakke (Hausa), Okwukwo-iri nwere-omughor (Ikwerre) in Nigeria [8]. However, some plants in the genus *Cissus* such as *Cissus sycoidesis* and *Cissus quadrangularis* have been reported to have antidiabetic and anti-hyperlipidaemic activity [9,10]. This present study examined the antidiabetic potential and the antihyperlipidaemic effects of ethanol extract of *C. gracillis.*

METHODS

Collection of plant materials

The plant *C. gracillis* was collected in Obelle Town, Emohua Local Government Area of Rivers State, Nigeria. Authentication was done by Dr. Ekeke Chimezie of the Department of Plant Science and Biotechnology, University of Port Harcourt with the herbarium number UPH/V/1249.

Plant extract preparation

The plant *C. gracillis* was collected, cleaned, shade dried for 2 weeks, and pulverized using a mechanical grinder. The plant powder (3.7 kg) was subjected to solvent extraction for 72 h with 17.84 L of absolute ethanol. The extract was concentrated using rotary evaporator and carefully evaporated to dryness over a water bath at 45°C while the dried extract was stored in a refrigerator.

Animals used

A total of 47 healthy albino rats of average weight of 200 g was used. The animals were procured from the Department of Pharmacology, acclimatized, and fed with standard feeds (Top feed broiler finisher manufactured by Premier Feed Mills Company limited, Cross Rivers State, Nigeria) and water *ad-libitum* under hygienic condition.

Phytochemical screening test

Preliminary screening of the ethanol crude extract of *C. gracillis* was carried out to determine the presence of its constituents [11]. The intensity of the coloration and precipitates formed determined the abundance of the compound present.

Acute toxicological evaluation

The acute toxicity study of the plant extract was determined using modified [12] Lorke's method with 18 healthy albino rats. The study was in two phases with 9 healthy albino rats each. In the first phase, the animals were randomly distributed to three groups of three animals each and treated with the ethanol extract of *C. gracillis* at doses of 10, 100, and 1000 mg/kg orally. The animals were kept under observation for 24 h for signs of acute toxicity and death.

In the second phase, three groups of three healthy albino rats each were administered with the ethanol extract of *C. gracillis* at doses of 1600, 2900, and 5000 mg/kg and were keenly observed for 24 h for signs of mortality and death.

Induction of diabetes mellitus

Diabetes was induced by intraperitoneal injection of alloxan monohydrate at a dose of 165 mg/kg body weight dissolved in cold normal saline. After a period of 72 h, the rats with fasting blood glucose levels >200 mg/dl were considered diabetic and thus selected for the study [13].

Evaluation of anti-diabetic and anti-hyperlipidaemic activity

The rats were allotted into seven groups of five rats each. Treatment was done daily for 21 days consecutively through oral gavage as outlined below:

1. Group A: Diabetic rats with glibenclamide (10 mg/kg)
2. Group B: Diabetic rats with atorvastatin (10 mg/kg)
3. Group C: Diabetic rats with *C. gracillis* extract (250 mg/kg)
4. Group D: Diabetic rats with *C. gracillis* extract (500 mg/kg)
5. Group E: Diabetic rats with *C. gracillis* extract (1000 mg/kg)
6. Group F: Diabetic untreated
7. Group G: Non-diabetic control.

Normal control and diabetic untreated animal groups received distilled water and standard feed. Body weights and the fasting blood glucose levels of all the animals were recorded at regular interval during the experimental period. Blood samples were collected from the tail tip of the animals on days 0, 7, 14, and 21, respectively. This was done with the aid of Accu-check glucometer test strips.

All the rats were sacrificed under anesthesia with diethyl ether at the end of the study and blood samples were analyzed for lipid profile parameters vis total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein at the Department of Chemical Pathology, the University of Port Harcourt with Randox kit (Randox lab. UK) and following the standard procedures as described by the manufacturers. The Low-density lipoprotein-cholesterol was calculated using Friedwald's equation [14,15]. Ethics approval was obtained from the University of Port Harcourt research ethics committee with the reference number UPH/RandD/REC/04.

Statistical analysis

All the results obtained were analyzed using one-way analysis of variance followed by Student's t-test with SPSS version 23. Comparison was done between treatment groups and control groups. p-values at $p<0.05$ were taken as significant.

RESULTS

Qualitative phytochemical screening

Qualitative phytochemical screening of the ethanol extract of the leaves and stem of *C. gracillis* revealed alkaloids, carbohydrates, cardenolides, and triterpenoids/steroids as its phytoconstituents while flavonoids, tannins, saponins, and anthraquinones were absent.

Acute toxicity

The result of the oral acute toxicity studies showed that the LD_{50} of the ethanol extract of *C. gracillis* was above 5000 mg/kg body weight. However, the main signs of acute toxicity observed was itching in the mouth which was highest with the dose of 5000 mg/kg.

Effect of the extract on blood glucose concentration

The extract at 500 mg/kg showed a statistically significant ($p<0.05$) decrease in blood glucose level when compared with the glibenclamide group on day 21 (Fig. 1).

Effect of the extract on lipid profile of albino rats

The extract at 125 mg/kg had a comparable total cholesterol value with the positive control group. The extract at 500 mg/kg exhibited a non-significant reduction in triglycerides level in comparison with the positive control and diabetic control groups. Treatment with the ethanol extract of *C. gracillis* showed a non-significant decrease in low-density lipoproteins at 125 mg/kg when compared with the diabetic untreated group (Fig. 2).

DISCUSSION

Diabetes mellitus which is a chronic metabolic syndrome is associated with abnormally high blood glucose level as a result of insufficient secretion or resistance to insulin. This study evaluated the potential antidiabetic and anti-hyperlipidemic activities of ethanol extract of the leaves and stem of *C. gracillis*. At least a dozen plants from the genus *Cissus* have been used for treating various ailments in traditional medicines in different parts of the world. The qualitative phytochemical screening of the extract showed the presence of alkaloids, carbohydrates, triterpenoids, and cardenolides. This result can be related to studies done by Deokule and Waghmare [16], which showed that the phytochemical screening of *C. quadrangularis* and *C. sycoidesis* contained sterols/triterpenoids, stilbens, iridoids, and had anti-diabetic and antilipidemic activities [17-19]. The oral acute toxicity of the ethanol extract of leaves and stems of *C. gracillis* showed only a mild itching in the mouth which was highest at the dose of 5000 mg/kg without any other adverse effect or mortality even at 5000 mg/kg, it could be said that the plant is very safe [12]. This result can be correlated to the report of Enechi [20], that the oral LD_{50} of the ethanol extract of the root of *C. quadrangularis* was greater than 5000 mg/kg.

Alloxan monohydrate causes enormous reduction in insulin release through the destruction of beta cells of the Islet of Langerhans, thereby inducing hyperglycemia [21]. *In vitro* studies have shown that alloxan monohydrate is selectively toxic to pancreatic beta cells causing cell necrosis. The cytotoxic action of alloxan monohydrate is mediated by the production of reactive oxygen species with resultant fragmentation of DNA of pancreatic cells [22]. In this study, the extract at 500 mg/kg showed a statistically significant ($p<0.05$) decrease in blood glucose level when compared with the glibenclamide group on day 21. However, gradual reduction in blood glucose levels was observed in the extract-treated groups on the 7^{th}, 14^{th} and 21^{st} days of treatment but could not considered to be significant. This result indicates a mild antidiabetic activity of the leaves and stem of *C. gracillis.* The mechanism by which *C. gracillis* may be eliciting its antidiabetic effect may be through the potentiation of pancreatic secretion of insulin from beta cells of islets of Langerhan or due to enhanced transportation and utilization of insulin in the peripheral tissues which can be corroborated with the reports of [23] on aqueous leaf extract of *Millettia aboensis*. The administration of ethanol extract of *C. gracillis* to alloxan-induced diabetic rats produced a decrease in total cholesterol, triglycerides,

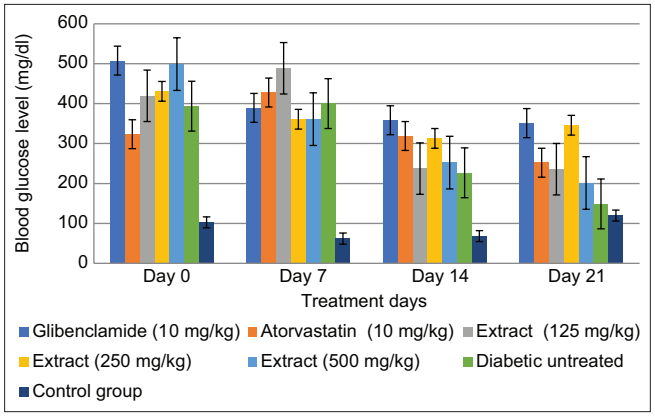

Fig. 1: Effect of *Cissus gracillis* on blood glucose concentrations of alloxan-induced diabetic rats

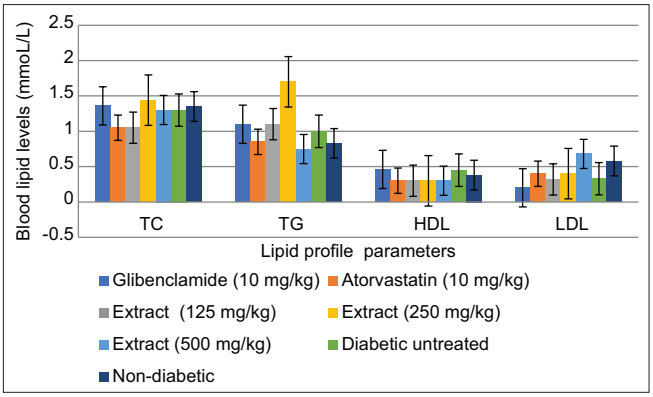

Fig. 2: Effect of *Cissus gracillis* on lipid profile of alloxan-induced diabetic rats, Values are represented as mean±SEM. TC: Total cholesterol, TG: Triglycerides, HDL: High density lipoprotein while LDL: Low density lipoprotein

and low-density lipoproteins which were comparable to glibenclamide and atorvastatin effects. This showed the protective effect of *C. gracillis* against the development of hyperlipidaemia. This can be related to the findings of [24] which stated that *Mimosa pudica* extract possessed anti-hyperlipidaemic effect. The antidiabetic and antihyperlipidaemic of *C. gracillis* may be attributed to the presence of alkaloids and triterpenoids as its phytoconstituents [25] while the absence of flavonoids and tannins in the extract may also be responsible for its inability to exhibit a pronounced antidiabetic activity [26].

CONCLUSION

The findings of this study have revealed that the ethanol extract of *C. gracillis* possess mild anti-diabetic activity and anti-hyperlipidaemic activity which may be due to the presence of alkaloids and triterpenoids as its biologically active constituents.

AUTHORS CONTRIBUTIONS

Author OAS conceptualized, designed, and supervised the study with the experimental work, while author GEIE carried out the study. Both authors drafted the manuscript while author AOS wrote the final manuscript.

CONFLICT OF INTEREST

The authors hereby declare that no conflict of interest exists.

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